FORENSIC TOXICOLOGY

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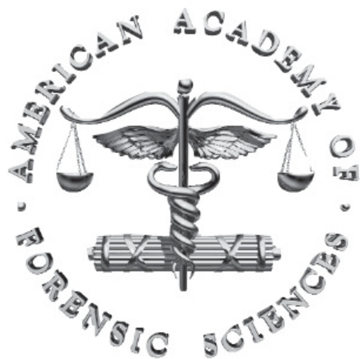
Forensic Toxicology



Forensic Toxicology

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Preface

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This further acknowledges the excellent work of all our Toxicoloy authors and colleagues worldwide who are willing to share their work for education

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Engineering Sciences

[1] [1] The Executive Committee of The American Academy of Forensic Sciences has directed Laura Liptai, Ph.D. of the Engineering Sciences Section to prepare this volume for publication.

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Analysis of Cocaine Analytes in Human Hair: Ultrastructural Evaluation of Human Hair by Microscopy for the Determination of Morphological Differences Following Surface Contamination and Laboratory Decontamination	*Jeri D. Ropero-Miller, PhD, RTI International, 3040 Cornwallis Road, PO Box 12194, Building 3, Room 116, Research Triangle Park, NC 27709; Peter R. Stout, PhD*, Kelly Shipkowski, BS, and John M. Mitchell, PhD, RTI International, Center for Forensic Sciences, RTI International, 3040 Cornwallis Road, Research Triangle Park, NC 27709-2194; Michael R. Baylor, PhD, Center for Forensic Sciences, RTI International, 3040 Cornwallis Road, Building 3, Research Triangle Park, NC 27709-2194; and Stacy Doorn, MS, and Owen Crankshaw, PhD, RTI International, 3040 Cornwallis Road, Johnson Building 105, Research Triangle Park, NC 27709*	**20**
Analysis of Cocaine Analytes in Human Hair II: Evaluation of Different Hair Color and Ethnicity Types Following Surface Contamination and Laboratory Decontamination	*Jeri D. Ropero-Miller, PhD*, and Cynthia Lewallen, MS, RTI International, 3040 Cornwallis Road, Building 3, 3040 Cornwallis Road, Research Triangle Park, NC 27709; Nichole D. Bynum, MS, Center for Forensic Sciences, RTI International, 3040 Cornwallis Road, Building 3, Research Triangle Park, NC 27709; Meredith Meaders, BS, RTI International, 3040 Cornwallis Road, Building 3, Research Triangle Park, NC 27709; Jordan N. Espenshade, MSFS, 1420 Centre Avenue, Apartment 103, Pittsburgh, PA 15282; and John M. Mitchell, PhD, Michael R. Baylor, PhD, and Peter R. Stout, PhD, Center for Forensic Sciences, RTI International, 3040 Cornwallis Road, Research Triangle Park, NC 27709*	**21**
Investigation of the Effect of Vinegar on Oral Fluid Drug Testing: Effects on Immunoassay Screening	*Eva M. Reichardt, MSc*, Bournemouth University, Talbot Campus, Fern Barrow, Poole, BH12 5BB, UNITED KINGDOM; Dene Baldwin, PhD, Concateno, 92 Milton Park, Oxfordshire, Abingdon, OX14 4RY, UNITED KINGDOM; and Michael D. Osselton, Bournemouth University, Fern Barrow, Talbot Campus, Poole, BH12 5BB, UNITED KINGDOM*	**21**
Drugs and Driving Special Scientific Session: Current Research Related to Drug-Impaired Driving	*Laura J. Liddicoat, BS, Wisconsin State Lab of Hygiene, Forensic Toxicology Section, 2601 Agriculture Drive, Madison, WI 53707-7996; Christine Moore, PhD*, Immunalysis Corporation, 829 Towne Center Drive, Pomona, CA 91767; Alain Verstraete, MD*, University Ghent, UZ Gent, De Pintelaan 185, Gent, B-9000, BELGIUM; James P. Zacny, PhD*, University of Chicago, Department of Anesthesia & Critical Care MC4028, University of Chicago, 5841 South Maryland Avenue, Chicago, IL 60637; and Amy K. Miles, BS*, Wisconsin State Laboratory of Hygiene, 2601 Agriculture Drive, PO Box 7996, Madison, WI 53707-7996*	**22**
Direct LC/MS/MS Quantification of Plasma Cannabinoids and Their Glucuronides	*David M. Schwope, MS*, National Institute of Heath, NIDA, 251 Bayview Boulevard, Suite 200, Room 05A721, Baltimore, MD 21224; Karl B. Scheidweiler, PhD, National Institute of Heath, NIDA/IRP, 251 Bayview Boulevard, Suite 200, Room 05A721, Baltimore, MD 21224; and Marilyn A. Huestis, PhD, Chemistry & Drug Metabolism, Intramural Research, National Institute of Heath, NIDA, 251 Bayview Boulevard, Room 05A721, Baltimore, MD 21224*	**22**

Cannabinoid Concentrations in Daily Cannabis Smokers' Oral Fluid During Prolonged Monitored Abstinence	*Dayong Lee, MS, and Allan J. Barnes, BS, National Institute on Drug Abuse, 251 Bayview Boulevard, Baltimore, MD 21224; Robert S. Goodwin, PhD, NIDA Intramural Research Program, Chemistry & Drug Metabolism Section, 251 Bayview Boulevard, Baltimore, MD 21224; Jussi Hirvonen, PhD, National Institute of Mental Health, Building 31, Room B2B37, 31 Center Drive MSC 2035, Bethesda, MD 20892-2035; and Marilyn A. Huestis, PhD*, National Institute on Drug Abuse, Chemistry & Drug Metabolism, Intramural Research, NIDA, NIH, 251 Bayview Boulevard, Room 05A721, Baltimore, MD 21224*	**23**
Effects of Smoking the Synthetic Cannabinoids JWH-018 and JWH-073 on Human Performance and Behavior: Controlled Administration and DUID Case Reports	*Amy Miles, BS, Wisconsin Laboratory of Hygeine, 465 Henry Mall, Madison, WI 53706; Barry K. Logan, PhD*, and Allan Xu, PhD, NMS Labs, 3701 Welsh Rd, Willow Grove, PA 18901; Robert Welsh, MS, University of Central Missouri, Missouri Safety Center, UCM Humphreys Building, Suite 200, Warrensburg, MO 64093; and Tracey Durbin, University of Central Missouri, UCM Humphreys Building, Suite 200, Warrensburg, MO 64093*	**24**
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Postmortem Pediatric Toxicology	*Robert A. Middleberg, PhD, NMS Labs, 3701 Welsh Road, Willow Grove, PA 19090; J. Scott Denton, MD*, McLean County Coroner's Office, 104 West Front Street, Bloomington, IL 61702-2400; Laureen Marinetti, PhD*, Montgomery County Coroner's Office, Miami Valley Regional Crime Lab, 361 West 3rd Street, Dayton, OH 45402; C. Andrew Robinson, PhD*, University of Alabama, Division, Laboratory Medicine, Department of Pathology, Birmingham, AL 35233-7331; and Thomas A. Andrew, MD*, Office of the Chief Medical Examiner, 246 Pleasant Street, Suite 218, Concord, NH 03301*	**28**

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Alcohol Elimination Rate Variability and Subject-Altered General Consumption of Alcohol	*Michael R. Corbett, PhD*, and Brittney R. Henderson, BSc, 7309 Sandhurst Drive, Mississauga, Ontario L5N 7G8, CANADA*	**30**
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A Retrospective Comparison of Blood- and Breath-Alcohol Results in Wisconsin Impaired Drivers 2001-2007	*Patrick M. Harding, BS*, Wisconsin State Laboratory of Hygiene, Toxicology Section, PO Box 7996, Madison, WI 53707-7996; and Rod G. Gullberg, MPA, Washington State Patrol, 811 East Roanoke, Seattle, WA 98102*	**32**
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Gastric Fentanyl Concentrations in Fentanyl-Related Deaths: A Study of 11 Cases	*Ruth E. Kohlmeier, MD*, Werner Jenkins, MS, MPA, Robert C. Bux, MD, Jennifer M. Hoffman, MS, and Christopher B. Clarke, BS, El Paso County Coroner's Office, 2743 East Las Vegas Street, Colorado Springs, CO 80906; and Andrea N. Phelps, and Emily D. Morrison, BS, Univeristy of Colorado at Colorado Springs, 1420 Austin Bluffs Parkway, Colorado Springs, CO 80918*	**33**
Cocaine Detection in Postmortem Samples Following Therapeutic Administration	*Kristen M. Bailey, MS*, David J. Clay, BA, and Myron A. Gebhardt, MS, Office of the Chief Medical Examiner, 619 Virginia Street West, Charleston, WV 25302; Matrina J. Schmidt, MD, West Virginia University, Department of Pathology, PO Box 9203, Morgantown, WV 26506; and James C. Kraner, PhD, Office of the Chief Medical Examiner, 619 Virginia Street West, Charleston, WV 25302*	**33**
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A HPLC/MS/MS Method for Simultaneous Determination of Three Opiates and Three Benzodiazepines in Postmortem Blood	*Shaohan Zhao, PhD*, Terry Danielson, PhD, and Ashraf Mozayani, PhD, PharmD, Harris County Medical Examiner's Office, 1885 Old Spanish Trail, Houston, TX 77054*	**38**
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Death Caused by Fentanyl Smoking	*Kristen M. Bailey, MS, David J. Clay, BA, Myron A. Gebhardt, MS*, James A. Kaplan, MD, and James C. Kraner, PhD, Office of the Chief Medical Examiner, 619 Virginia Street, West, Charleston, WV 25302; and Carrie J. Kirkpatrick, BS, West Virginia State Police, 725 Jefferson Road, South Charleston, WV 25309*	**40**
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Workplace Toxicity In the Archives of Ottoman Empire	*Salih Cengiz, PhD*, and Selda Mercan, MS, Istanbul University, Institute of Forensic Science, Cerrahpasa, Istanbul, 34303, TURKEY; T. Mehmet Karayel, BS, Istanbul University, Institute of Forensic Sciences, Istanbul Universitesi, Adli Tip Enstitusu, Cerrahpasa Kampusu, PK.10, 34303, Istanbul, 34303, TURKEY; and Zeynep Turkmen, MS, Istanbul University, Institute of Forensic Sciences, Cerrahpasa, Istanbul, 34303, TURKEY*	**44**
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You Don't Look/You Don't See: Delayed Death Due to Suboxone Ingestion Involving Analysis of Alternate Non-Biological Specimens (Clothing) – The Cleveland Experience	*Elizabeth K. Balraj, MD*, Eric S. Lavins, BS, and Curtiss L. Jones, MS, Cuyahoga County Coroner's Office, 11001 Cedar Avenue, Cleveland, OH 44106; Lee M. Blum, PhD, NMS Labs, Inc., 3701 Welsh Road, Willow Grove, PA 19090; and Deborah Y. Shaffer, BS, Laura D. Wilkins, BS, Mohammed Sarwar, PhD, and Frank P. Miller III, MD, Cuyahoga County Coroner's Office, 11001 Cedar Road, Cleveland, OH 44106*	**48**
NMR Analysis of 3,4-methylenedioxy-Nmethylamphetamine (MDMA or Ecstasy) and its Metabolites in Urine	*Elise Champeil, PhD*, John Jay College of Criminal Justice, 445 West 59th Street, New York, NY 10019; Gloria Proni, PhD, John Jay College of Criminal Justice, Science Department, 445 West 59th Street, New York, NY 10019; and Jonathan Liu, MSc, John Jay College, 445 West 59th Street, New York, NY, 10019*	**48**
Applications of Fire Debris Analysis to Problems in Toxicology	*Wayne Moorehead, MSc*, and Ines Collison, PhD, Orange County Sheriff-Coroner, 320 North Flower Street, Santa Ana, CA 92703*	**49**
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Propofol Analytical Challenges and Interpretation	*Sherri L. Kacinko, PhD*, and Barry K. Logan, PhD, NMS Labs, 3701 Welsh Road, Willow Grove, PA 19090*	**50**
Detection of Fentanyl and Lidocaine in Dried Blood Spots Using High Performance Liquid Chromatography Tandem Mass Spectrometry	*Roman Karas, BS*, FBI Laboratory, 2501 Investigation Parkway, Chemistry Unit, Quantico, VA 22135-0001*	**51**

Postmortem Pediatric Toxicology	*Robert A. Middleberg, PhD, NMS Labs, 3701 Welsh Road, Willow Grove, PA 19090; Nikolas P. Lemos, PhD, San Francisco Office of the Chief Medical Examiner, Hall of Justice, North Terrace, 850 Bryant Street, San Francisco, CA 94103; Wendy R. Adams, PhD*, NMS Labs, Inc., 3701 Welsh Road, Willow Grove, PA 19090; Margaret Greenwald, MD*, Office of Chief Medical Examiner, Maine, 37 State House Station, Augusta, ME 04333; Eric L. Kiesel, MD, PhD*, Pierce County Medical Examiner's Office, 3619 Pacific Avenue, Tacoma, WA 98418; and Loralie J. Langman, PhD*, Hilton 761-B, 200 1st Street, Southwest, Rochester, MN 55905*	**51**
Melendez-Diaz and Other 6th Amendment, Confrontational Clause Cases - Their Impacts and Perspectives	*Peter R. Stout, PhD, Center for Forensic Sciences, RTI International, 3040 Cornwallis Road, Research Triangle Park, NC 27709; Roderick T. Kennedy, JD*, New Mexico Court of Appeals, PO Box 25306, Albuquerque, NM 87504-2008; Robert A. Middleberg, PhD*, NMS Labs, 3701 Welsh Road, Willow Grove, PA 19090; Corey L. Maze, JD*, State of Alabama, 500 Dexter Avenue, Montgomery, AL 36130; and Richard D. Friedman, DPhil, JD*, University of Michigan Law School, 625 South State Street, Ann Arbor, MI 48109*	**52**
Brain Serotonin Transporter Reduction in Human Polydrug MDMA (Ecstasy) Users	*Stephen J. Kish, PhD*, Human Neurochem Pathology Lab, Addiction & Mental Health, 250 College Street, Toronto, ON M5T 1R8, CANADA; Yoshiaki Furukawa, MD, PhD, Juntendo Tokyo Koto Geriatric Medical Center, 3-3-20 Shinsuna, Koto, Tokyo, 136-0075, JAPAN; Diana G. Wilkins, PhD, Center for Human Toxicology, Biomed Research Polymers Building, Room 490, Salt Lake City, UT 84112; and Isabelle Boileau, PhD, Centre for Addiction and Mental Health, 250 College Street, Toronto, ON M5T1R8*	**52**
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The Analysis of PM Oral Swabs by SPE and LC–MSMS for Fentanyl as an Indicator of Administration	*Jeffery Hackett, MSc*, Northern Tier Research, 1300 Old Plank Road, Mayfield, PA 18433; Albert A. Elian, MS, Massachusetts State Police Crime Laboratory, 59 Horse Pond Road, Sudbury, MA 01776; and Robert J. Osiewicz, PhD, Erie County Medical Examiners Laboratory, 462 Grider Street, Buffalo, NY 14125*	**55**
Rapid Quantification of THC and Carboxy–THC in Blood by LC–MS/MS	*Albert A. Elian, MS*, Massachusetts State Police Crime Lab, 59 Horse Pond Road, Sudbury, MA 01776*	**55**
Simultaneous Quantification of Twenty Common Drugs of Abuse and Metabolites in Human Meconium by LCMSMS	*Teresa R. Gray, MS*, National Institute on Drug Abuse, Chemistry and Drug Metabolism, IRP, NIH, 251 Bayview Boulevard, Suite 05A406, Baltimore, MD 21224; Diaa M. Shakleya, PhD, National Institute on Drug Abuse, 251 Bayview Boulevard, Suite 05A729.02, Baltimore, MD 21224; and Marilyn A. Huestis, PhD, National Institute on Drug Abuse, Chemistry & Drug Metabolism, Intramural Research, National Institute on Drug Abuse, NIH, 251 Bayview Boulevard, Suite 05A721A, Baltimore, MD 21224*	**56**
Analysis of Ethylglucuronide (EtG) and Ethylsulfate (EtS) in Urine by Liquid Chromatography Mass Spectrometry	*Sandra Valtier, PhD*, Clinical Research Division, 2200 Bergquist Drive, Building 4430, Lackland AFB, TX 78236*	**57**
Validation of Opiate Detection and Quantification in Human Urine Using Liquid Chromatography and Tandem Mass Spectrometry (LC/MS/MS)	*Chelsy L. Wingate, BS*, 1517 Hawk Tree Drive, College Station, TX 77845*	**57**
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Nine Xylazine Related Deaths in Puerto Rico	*Carlos F. Chavez-Arias, MD*, Puerto Rico Institute of Forensic Sciences, PO Box 11878, Caparra Heights Station, San Juan, Puerto Rico 00922-1878; Dario Sanabria, MD, Puerto Rico Institute of Forensic Sciences. Department of Pathology, PO Box 11878, Caparra Heights Station, San Juan, Puerto Rico 00922-1878; and Kazandra Ruiz, BS, and Jose Rodriguez-Orengo, PhD, Puerto Rico Institute of Forensic Sciences, PO Box 11878, San Juan, Puerto Rico 00922-1878*	**77**
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TOXICOLOGY

CHICAGO 2011

K1 Analysis of Amphetamine on Swabs and Oral Fluid Sampling Device: A SPE Approach

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After attending this presentation, attendees will learn about the extraction of amphetamine from an oral swab and an oral fluid sampling device using readily available solid phase extraction (SPE) cartridges and tandem mass spectrometry. Use of this SPE method will permit analysts to compare results from both types of sampling methods.

This presentation will impact the forensic science community by offering analysts in forensic toxicology data from methods that permit samples of oral fluid material to be analyzed in a clean format with minimal matrix effects and excellent analytical characteristics in terms of both SPE and LC-MS/MS.

Method: Extraction (SPE) was performed on mixed mode column (C_8/SCX) conditioned with methanol, deionized water, and pH 6 buffer (3 mL, 3 mL, and 1 mL, respectively) prior to sample loading. Oral samples (swabs/ fluid sampling device) were taken 1 hour after administration of prescribed amphetamine. The swabs were extracted with methanol and adjusted to pH 6 with 0.1 M phosphate buffer (5 mL). The samples from the sampling device were extracted into 3 mL of a proprietary formulated buffer (pH 7) containing a non-azide preservative. A 1 mL aliquot was buffered with 5 mL of 0.1 M phosphate buffer. To both sets of sample an internal standard was added (amphetamine-d_5). After loading the sample, the sorbent was washed with deionized water, acetic acid, and methanol (3 mL of each, respectively). Each SPE column was eluted with 3 mL of a solvent consisting of dichloromethane-isopropanol-ammonium hydroxide (78:20:2). An aliquot of this solvent was treated (details presented) with the mobile phase and analyzed by LC-MS/MS in positive multiple reaction monitoring (MRM) mode. Data is presented for MRM's of amphetamine and the internal standard, respectively.

Liquid chromatography was performed in gradient mode employing a 50 mm x 2.0 mm C_{18} analytical column and a mobile phase consisting of acetontitrile and 0.1% aqueous formic acid. The gradient was programmed to run from 5% to 90% acetonitrile in 4.0 minutes and then back to 5% for re-injection. The total run time for each analysis was less than 5 minutes. In this presentation, representative chromatograms are shown to illustrate the efficiency of the chromatography and analysis.

Results: The limits of detection/quantification for this method were determined to be 5 ng/ mL and 10 ng/ mL, respectively for amphetamine. The method was found to be linear from 10 ng/ mL to 500 ng/ mL (r^2>0.999). Data is presented to show that recovery of amphetamine was found to be > 94 %. Interday and Intraday analysis of amphetamine were found to be < 4% and < 6%, respectively. Matrix effects were determined to be < 4%. Analysis of the subject swab concentrations ranged from 16 to 129 ng/ mL (mean: 46 ng/ mL (sample size =10)), while the oral sampling device ranged from 52 to 846 ng/ mL (mean: 132 ng/ mL (sample size =10)).

Conclusion: The use of this procedure for the analysis of amphetamine adds to the body of knowledge regarding the analysis of amphetamine. The data should be of great use to analysts in the field of forensic toxicology employing oral fluid analysis, as it demonstrates how far the horizons of oral fluid sampling can be expanded as it permits a direct comparison between oral swabs and oral sampling devices in relation to the analysis of amphetamine after oral administration. The novelty of this study is the originality of the compare and contrast approach (as demonstrated by the presented data) to the analysis of amphetamine using readily available swabs and commercially available oral fluid devices. This limited study indicates the range of concentrations of the drug that can be achieved using either system.

Amphetamine, SPE, LC-MS/MS

K2 The Effect of Drugs and Alcohol on Autopsy Cases Performed at the William L. Jenkins Forensic Center From 2003-2009

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After attending this presentation, attendees will have an appreciation of the effect of drugs and alcohol in the autopsy cases for Upper East Tennessee performed at the William L. Jenkins Forensic Center from 2003 through 2009.

This presentation will impact the forensic science community by providing descriptive statistics on the impact of alcohol and/or drugs and determining whether any exist in the autopsies performed from 2003 through 2009.

The William L. Jenkins Forensic Center has performed autopsies on questionable and medico-legal deaths which occurred in the eight counties of the First Tennessee Development District from 2003 through 2009. The purpose of this research was to compile descriptive statistics on the impact of alcohol and/or drugs, and determine whether any trends exist in the autopsies performed from 2003 through 2009. Toxicological evaluations of specimens collected at autopsy were used to determine if drugs and/or alcohol were involved in the deaths. A descriptive database was established defining all parameters and data pertinent in each case (age, sex, cause/manner of death, and toxicological results). Specimens (blood, gastric contents, urine, and vitreous humor) from the autopsies were analyzed for drugs and alcohol using multiple analytical toxicological procedures including: colorimetric, thin layer chromatography (TLC), immunochemistry, gas chromatography (GC), gas chromatography mass spectroscopy (GCMS), and liquid chromatography mass spectroscopy (LCMS). Toxicological results were compiled in an electronic database to allow for analysis and interpretation. Case number per year ranged from a minimum of 226 (2004) to a maximum of 306 (2009) with a general increase in the number of cases per year over the period. Results indicate that the impact of alcohol and drugs as a percentage of cases ranged as follows: positive for drugs from 76% (2009) to 87% (2003), positive for drugs and alcohol from 19% (2009) to 33% (2003), and positive for alcohol alone from 22% (2009) to 36% (2004). Acute drug overdose was the cause of death in 22% (2009) to 35% (2007) of cases per year. While the percentages of cases with drugs, drugs and alcohol, and alcohol alone varied from year to year, the proportionality of these groups to one another remained relatively constant over the years analyzed. In the

 * Presenting Author

range of years studied, drugs appeared in a greater percentage of cases than drugs and alcohol, which appeared in a greater percentage of cases than alcohol alone. The most prevalent groups of drugs present at autopsy, other than alcohol, were opiates and benzodiazepines. These drugs were present in ranges from 14% (2005) to 26% (2007). Other major drugs, or classes of drugs present (as a percentage of case per year) were: cocaine from 6% (2009) to 15% (2005), methadone from 6% (2003 and 2009) to 12% (2006 and 2007), stimulants from 7% (2003 and 2009) to 16% (2005 and 2006), and sedatives from 5% (2004) to 12% (2003 and 2006). There was an increased prevalence of opiates and benzodiazepines in our forensic cases from 2005 through 2009, as well as an increase in the number of autopsy cases in which these drugs were found in combination. This increase may reflect the amplified clinical use of these drugs in our region, misuse of prescription drugs, or increased diversion of prescription medications.

Toxicology, Alcohol, Autopsy

K3 Measurement Uncertainty of GC Method in Determining Ethanol Concentration of In-House Prepared Aqueous Standards for use with Evidentiary Breath Test Instruments

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After attending this presentation, attendees will better understand the principles of ISO "Guide to the Expression of Uncertainty in Measurement" as applied to a specific gas chromatography (GC) method for ethanol chemical measurement. This paper defines the measurement problem, describes the evaluation steps involved, shows the largest sources of uncertainty, and demonstrates how the formal process led to changes to the method that resulted in achieving a combined uncertainty goal.

This presentation will impact the forensic science community by giving an example of how to determine and express the confidence of results as a combined uncertainty based on traceability to a certified reference material (CRM).

The objective of this work was to establish the uncertainty of the concentration of ethanol in aqueous solution standards prepared by our laboratory. The principle goals of this study were: (1) to estimate the combined standard uncertainty of in-house standard solution using a GC method; and (2) to see if it was possible to develop a new GC method with tighter quality controls than available with the present method.

The measurement was the mass concentration of ethanol in water in which the concentration is a function of the uncertainty sources of the batch sampling, the GC method, which included the entire sequence of steps from sample auto-dilution through final GC analysis, the uncertainty of the certified reference material used in determining the linear calibration slope and the uncertainty of the calibration least squares fit process.

The measurement problem was a concentration of ethanol analyte in single sample matrix with a range of analyte concentrations. The GC measurement is calibrated against traceable CRMs. Because the method has been under statistical control, the precision information from previous runs includes the *combined* effect of nearly all of the potential sources of uncertainty. Precision estimates used were over an extended period of time, by different analysts using different equipment and the replicate analysis of several samples was the choice for precision data.

The precision of the measurement was found to vary both proportionally with analyte concentration level (level dependant contributions of analyte) and a constant related to the calibration regression method that is independent of analyte concentration. The three largest sources of uncertainty were determined to be Calibration Slope (1.2%), GC Analytical (0.6%), and CRM (0.7%). The Combined Standard Uncertainty was calculated as U = 1.6% (95% C.L., K=2).

The results show that the proposed method is suitable to expect GC calibrators (ones above 0.098 g/100ml) to be within 2% (k=2) of their target value vs. the present method of 5%; and the sample solution measurement to be within 2% (k=2) of target vs. the present method of 10% of QCs. Below 0.098 g/100ml concentration, the acceptance criteria will need to be established that is not based on a percentage since the uncertainty is not linearly proportionally to concentration in the extremes.

Based on this study, routine 0.080 g/210L and 0.160 g/210L breath alcohol standard solutions are estimated to have a combined standard uncertainty of < 2 % at a 95% confidence limit.

Measurement Uncertainty, Standards, Gas Chromatography

K4 Prevalence of Norhydrocodone in Authentic Hydrocodone Urine Specimens

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The goal of this presentation is to evaluate the incidence and disposition of norhydrocodone in authentic urine specimens of pain patients prescribed hydrocodone.

This presentation will impact the forensic science community by demonstrating applicability of norhydrocodone for the reliable confirmation of hydrocodone positive urine samples.

Hydrocodone is a common semi-synthetic opiate used as an antitussive and an analgesic.[1] Hydrocodone is excreted in urine as unchanged drug (49.8%) and metabolites: norhydrocodone (20.7%), conjugated hydromorphone (16.6%), 6-hydrocodol (12.4%), and conjugated 6-hydromorphol (0.4%).[1] The high potential for hydrocodone abuse may be due to the relative ease of purchase and the prevalence of use among chronic pain patients. Urine drug testing of pain patients for such drugs plays a pivotal role in the management of their prescribed medication. Monitoring of drug adherence, possible drug abuse, and diversion of prescribed drugs should be considered. Opiate urinalysis has been challenged by complex biotransformation of parent drugs and the commercial availability of the metabolites, such as hydromorphone.[2] Therefore, it is necessary to detect distinctive biomarkers for more accurate interpretation in the absence of parent drug. Normetabolites are considered unique metabolites indicating the ingestion of parent drugs. This study aims to detect norhydrocodone in urine specimens of pain management patients prescribed hydrocodone.

Authentic urine specimens (n=101) from pain management patients prescribed hydrocodone were obtained. Norhydrocodone was incorporated into the current opiate assay, which includes codeine, hydrocodone, hydromorphone, morphine, noroxycodone, oxycodone, and oxymorphone. The absence of co-elution effects between norhydrocodone and the other opiates was confirmed. The new method was validated to verify the reliability of norhydrocodone detection and quantification. The concentrations of norhydrocodone, hydrocodone, and hydromorphone in urine samples were measured. Urine specimens were treated with acid for the hydrolysis of conjugated glucuronide moiety and then injected into a liquid chromatograph tandem mass spectrometer (LC/MS/MS) equipped with columns utilizing turbulent flow technology.

The limits of detection and quantification (LOD and LOQ) and the upper limit of linearity (ULOL) for norhydrocodone were 100ng/mL,

250ng/mL and 100,000ng/mL, respectively. Intraday and interday precision and accuracy were conducted at 300, 3000, 30000ng/mL and showed <14.4% coefficient of variation and <±14.7% deviation from the target concentrations. Of the total urine specimens, 90.1% were positive for norhydrocodone, demonstrating that it is a common metabolite in hydrocodone urine specimens. Urine specimens containing norhydrocodone alone totaled 3%. The mean relative abundances of hydrocodone, norhydrocodone, and hydromorphone in the urine samples were 31.1%, 62.4% and 11.8%, respectively. This is inconsistent with previous reports showing unchanged parent drug as the major analyte present in urine after metabolism of hydrocodone. The results imply that the chronic use of hydrocodone increases the abundance of norhydrocodone metabolite in urine compared to single-dose usage.

Norhydrocodone is a prevalent and dominant metabolite in urine following the consumption of hydrocodone by chronic pain patients. It is a unique biomarker that can provide more conclusive confirmation and to a lesser extent reduce false negatives in urine drug testing for hydrocodone.

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Norhydrocodone, LC/MS/MS, Prevalence

K5 Self-Administration of Anesthetic (Propofol and Midazolam) and Psychotropic (Amitriptyline and Zolpidem) Drugs: Recreational Abuse and Suicidal Manner in an Anesthetist

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After attending this presentation, attendees will understand that forensic investigations are based on a multidisciplinary approach in which autopsy findings and toxicological results often result in association with circumstances and crime scene investigations.

This presentation will impact the forensic science community by demonstrating how recreational abuse of anesthetic and sedative agents in health care practitioners, especially anesthesiologists is an increasing event. This presentation will also show an unusual case of suicide in which the manner and means of suicide was dependent upon the occupation of the victim.

Presented is a case of a 50-year-old man, anesthetist at the main local hospital, who was found dead in the house where he lived alone since separating from his wife. On the previous evening he was found on the landing's floor with an occipital bruise injury and treated in the Emergency Department. The next day his brother, alerted by his colleagues that tried in vain to contact him, went to his house. He found that the front door had been left ajar, with a piece of furniture behind it. When he entered the flat, he noticed the corpse of the brother, supine on the living room's floor near a piece of furniture. There were two drips with intravenous tubes almost empty (approximately 1 ml). One drip was still inserted in the dorsum of the victim's right hand with tube for intravenous drip totally open. On the glass of this drip there was written "Miclela Caput" (meaning "Caput Mixture," written incorrectly). On the glass of the other drip there was written "500 TPS+200 DIPR" (meaning Sodium Thiopental+Diprivan). In the house there were some empty blisters of Zolpidem, more than 20 packs of different drugs (some of them empty), an ash-tray containing white liquid, several empty ampoules of Propofol, Midazolam and Thiopental, and several new and used syringes. In the bedroom there were two knapsacks containing pornographic materials and four plastic phalli.

External examination revealed abundant livor mortis, numerous recent needle marks with fresh and older hemorrhages in both arms, and a sutured occipital injury.

Autopsy and histological findings were pulmonary and brain oedema, moderate fatty liver, acute poly-visceral congestion, hemorrhagic pancreatitis.

Systematic toxicological analysis was performed on biological and non biological samples for alcohol, drugs of abuse and pharmaceuticals.

Blood toxicological examination by GC/MS revealed lethal concentration of Zolpidem (0.86 µg/ml) and high therapeutic blood concentrations of Propofol (0.30 µg/ml), Midazolam (0.08 µg/ml), Amitriptyline (0.07 µg/ml), and low concentration of Thiopental (0.03 µg/ml). Zolpidem was also found in gastric content while Thiopental was found in urine. Hair segment analysis (0 – 2 cm) revealed Propofol (4,7 µg/mg) and the presence of Zolpidem, Amitriptyline and Ketoprofen.

Residual's toxicological analysis of the inserted drip ("Caput mixture") revealed Propofol and Midazolam (approximately 1,9 and 0,08 mg/ml). Analysis of the non-inserted drip, showed Propofol and Thiopental (approximately 2 and 5 mg/ml). The low blood concentration of Thiopental suggests a self administration of the non-inserted drip at least 12 hours before death.

The blood Propofol level was lower than or within the commonly accepted therapeutic range of 1.3– 6.8 µg/ml after a standard anesthetic induction dose. Published reports indicate that in most cases, the postmortem Propofol concentrations were at therapeutic levels. It should be pointed out that especially for those agents used in anesthesia; the therapeutic concentrations refer to patients being supported respiratory-wise, while in non-supported or non-intubated patients such concentrations may be lethal. Most of those deaths are thought to have occurred because of the rapidity of Propofol's injection which led to apnea and death. A mere interpretation of the blood and tissue concentrations of Propofol in the toxicological analysis may be of limited diagnostic significance without taking into account the before mentioned reports. Toxicological analysis of hair confirmed the recreational abuse of Propofol.

These anesthetic and sedative drugs are often used in combination for anesthesia's induction. All of these act synergistically in combination and may induce respiratory depression. This effect depends on individual susceptibility, on dose used and, especially for Propofol and Midazolam, infusion's rapidity.

In conclusion, the victim was administered a solution of anesthetic drugs, rapidly infused in a lethal combination and simultaneously a hypnotic drug in lethal dose.

Anesthetic Drugs, Propofol, Suicide

K6 Simultaneous Detection of Psychedelic Amphetamines in Urine By LC/MS/MS

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After attending this presentation, attendees will be familiar with a technique for the simultaneous detection of eleven designer amphetamines in urine by liquid chromatography/tandem mass spectrometry (LC/MS/MS).

This presentation will impact the forensic science community by providing a new method for the simultaneous detection and quantitation of emerging drugs of abuse in urine.

Psychedelic amphetamines are a relatively new class of designer drug in the United States. These drugs were initially popular in Europe and Asia, but the 2C-, 2CT- and DO- series of amphetamines are now routinely seized throughout the United States. A number of these substances are not scheduled in the Federal Controlled Substances Act, offering users a legal alternative to the more traditional designer amphetamines like 3,4-methylenedioxymethamphetamine (MDMA). Many of these newer designer amphetamines produce profound hallucinogenic effects due to their structural similarity towards both mescaline and amphetamine. The pharmacology and toxicology of these drugs are considerably less studied than their conventional counterparts. Their prevalence among toxicological casework in the U.S. is not known, but many toxicology laboratories do not screen for these substances.

The drugs included in this study were 4-bromo-2,5-dimethoxyphenethylamine (2C-B), 4-methylthioamphetamine (4-MTA), 2,5-dimethoxy-4-ethylamphetamine (DOET), 2,5-dimethoxy-4-iodoamphetamine (DOI), 2,5-dimethoxy-4-methylamphetamine (DOM), 2,5-dimethyoxy-4-ethylthiophenethylamine (2C-T-2), 2,5-dimethyoxy-4-(i)-propylthiophenethylamine (2C-T-4), 2,5-dimethoxy-4-(n)-propylthiophenethylamine (2C-T-7), 2,5-dimethoxyphenethylamine (2C-H), 2,5-dimethoxy-4-iodophenethylamine (2C-I), and 2,5-dimethoxy-4-bromoamphetamine (DOB). A positive electrospray ionization (ESI) LC/MS/MS procedure was developed to allow simultaneous detection and quantitation of these substances following solid phase extraction (SPE).

Negative urine was fortified with the drugs of interest and extracted using SPE. In the absence of commercially available deuterated analogs, mescaline-d9 was chosen as the internal standard. An alkaline extraction using a copolymeric mixed-mode SPE column was used to isolate the drugs. Optimal drug recoveries were achieved using 2% ammonium hydroxide in 95:5 v/v methylene chloride:isopropanol. Separation was achieved using a C18 LC column and gradient elution (5% methanol in 50mM ammonium acetate and 100% acetonitrile in 50mM ammonium acetate). The total run time was approximately 5 minutes. Data was acquired using the following ions (precursor ions are underlined): m/z <u>262</u>, 245, 230 for 2C-B; m/z <u>182</u>, 165, 117 for 4-MTA; m/z <u>224</u>, 207, 179 for DOET; m/z <u>322</u>, 305, 105 for DOI; m/z <u>210</u>, 193, 178 for DOM; m/z <u>242</u>, 225, 134 for 2C-T-2; m/z <u>256</u>, 239, 197 for 2C-T-4; m/z <u>256</u>, 239, 197 for 2C-T-7; m/z <u>182</u>, 165, 150 for 2C-H; m/z <u>308</u>, 291, 276 for 2C-I; m/z <u>276</u>, 259, 231 for DOB. Limits of detection for all target analytes were 1-2 ng/mL and limits of quantitation were 1-6 ng/mL. Precision and accuracy was evaluated at 20 and 75 ng/mL. For all drugs, accuracy at 20 ng/mL was 95-115% and CVs were 4.3-10.0% (n=4). At 75 ng/mL accuracy was measured in the range 83-120% and CVs were 3.7-12.0 (n=4). Ion suppression and interferences from common amphetamines and endogenous phenethylamines were included in the study.

The technique allows for the simultaneous low-level detection of eleven psychedelic amphetamines in urine samples. The method will be further developed to determine the prevalence of these drugs in toxicological casework and to further develop a confirmation and quantitation procedure for these and other designer drugs in blood samples.

Designer Amphetamines, LC/MS/MS, Solid Phase Extraction

K7 Study of L-2-Aminothiazoline-4-Carboxylic Acid as a Biomarker for Cyanide Poisoning by LC-MS/MS Analysis

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After attending this presentation, attendees will understand how cyanide poisoning can be detected by measuring the amount of a derivative of cyanide, L-2-Aminothiazoline-4-Carboxylic Acid (ATCA) in biological fluids. ATCA can be easily measured in blood, urine, and organs from a subject.

This presentation will impact the forensic science community, as well as the Army, by improving the detection of cyanide from poisoning in various ways.

One threat of cyanide poisoning is the use of cyanide as a chemical warfare agent (CWA). Once exposure is identified, the amount of poison can be quantified and a more accurate treatment distributed. Identification of cyanide or its metabolites in biological fluids is necessary for many purposes in forensic, clinical, military, research, and veterinary fields. However, because of the volatility of cyanide and the difficulty of establishing steady-state cyanide levels with time, methods of directly evaluating cyanide levels are limited.

These studies focus on a chemically stable urinary metabolite of cyanide, 2-aminothiazoline-4-carboxylic acid (ATCA), which is an effective biomarker for cyanide exposure, specifically in mice liver samples. ATCA was used because it is stable over time, unlike cyanide, and its concentration level is directly proportional to the amount of cyanide from exposure. After using a method previously developed to dissect, preserve organs, and homogenize the livers, the organs were spiked with an internal standard, 2-aminothiazole-4-carboxylic acid (ATZA). The similarity between ATCA and ATZA is advantageous because ATZA is co-eluted with ATCA and detected at the same time by LC-MS/MS, therefore experiencing the same magnitude of ion suppression. ATCA was then extracted by solid phase extraction (SPE). Endogenous levels of ATCA were determined by comparing the non-exposed livers to calibrators containing known concentrations of ATCA, both of which were evaluated by the LC-MS/MS.

Mice were later exposed to various doses of cyanide and liver ATCA contents were compared to the dose of cyanide mice were given. An optimal method was developed to detect ATCA, with a recovery of 40-50%. Endogenous levels of ATCA in liver were found to be at least 100 ng/ml and were measured multiple times. This study indicates that this method can continue to be used for other organs, such as kidney, lung, and heart, to detect endogenous ATCA. Future studies will also concentrate on determining the concentration of ATCA in organs obtained from mice that were previously exposed to cyanide and compare the differences between endogenous ATCA levels and levels after exposure.

These studies were supported by the Army Medical Research Institute of Chemical Defense (Delivery Order 0878, Contract No. DAAD19-02-D-0001, TCN 06-170 and 08284) and the Robert A. Welch Foundation (x-0011) at Sam Houston State University, Huntsville, TX.

Cyanide, L-2-aminothiazoline-4-carboxylic acid (ATCA), Trace Evidence

K8 Purity of Street Ketamine Preparations Retrieved From Night Club Amnesty Bins in London

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The goals of this presentation are to describe the analysis of street ketamine in order to determine the purity of samples commonly available and to identify what impurities might be present.

This presentation will impact the forensic science community by showing how the majority of street ketamine samples analyzed were of high percentage purity suggesting that ketamine may be responsible for effects on the urogenital system. This also supports the observation that a number of patients undergoing clinical therapy with ketamine have reported similar symptoms.

Introduction: Ketamine has been widely used in medicine and veterinary practice for its anesthetic and analgesic properties linked with minimal respiratory depression. More recently the drug has gained popularity as a recreational substance amongst young people. Street prices of the drug vary between £10 and £20 per gram in the UK. The UK club magazine Mixmag survey of its readers in 2009 shows 51% used ketamine in last year, 32% in last month and 18% use it weekly. 30% experienced stomach pains after taking ketamine and 20% experienced urinary tract problems (more in women). A number of reports have appeared in the medical literature suggesting a possible link between ketamine misuse and kidney and bladder disorders. The pathological cause of the bladder related problems is at present unknown and it is uncertain whether they are attributable to ketamine or to impurities that may be present in street preparations. Little information is available concerning the purity of street ketamine hence analysis was undertaken on street preparations of the drug retrieved from amnesty bins in London night clubs. In this paper, the analysis of street ketamine is described to determine the purity of samples commonly available and to identify what impurities might be present.

Method: Street ketamine samples were analyzed using HPLC with diode-array in order to determine the percentage of ketamine present in the sample and identify any impurities. The system was equipped with a C_{18} reversed phase column which was maintained at 50°C. The mobile phase was a mixture of 5 mM SDS in 20 mM KH_2PO_4:acetonitrile (65:35, v:v) at a flow rate of 1.0 mL/min. In addition to HPLC analysis, samples were also analyzed using electron microscopy, color tests, FTIR with golden gate, GC-MS and TLC in an attempt to determine the nature of any impurities present.

Results: The purity of samples containing Ketamine only ranged between 65%—100% (mean = 87.9%; SD = 11.66%). Benzocaine was the principal impurity detected and ranged between 2.75%—16.60% (mean = of 7.27%; SD = 3.96%). Ketamine in samples containing Benzocaine ranged between 49.9% - 84% (mean = 67.21%; SD = 9.71%).

Conclusion: The majority of street ketamine samples were of high percentage purity suggesting that ketamine may be responsible for effects on the urogenital system. This also supports the observation that a number of patients undergoing clinical therapy with ketamine have reported similar symptoms.

Ketamine use is increasing rapidly worldwide and knowledge concerning the availability, purity, and trends in drug use can be of assistance to drug enforcement/legislation agencies as well as healthcare workers who may be involved in the provision of care to individuals following drug use. The results of this survey would support a hypothesis that bladder related diseases observed in ketamine users is likely to be attributable to ketamine rather than impurities or cutting agents.

Ketamine, Purity, Bladder Disorders

K9 Confirmation of Oleander Poisoning by LC/MS

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After attending this presentation, attendees will understand how to confirm oleander poisoning cases from blood and urine specimens.

This presentation will impact the forensic science community by providing the toxicological data necessary to make diagnostic decision about the patient when oleandrin is detected by toxicological screening.

In this case a 60-year-old woman was brought to emergency room with initial symptoms of vomiting, diarrhea, and abdominal pain. The patient's heart beat was normal at the beginning but then sinus bradycardia was observed gradually. Information obtained from her indicated that she is a cancer patient and that she drank the juice of some leaves of the oleander plant (*Nerium oleander L.* - Apocynaceae) for herbal self treatment. *Nerium oleander L.* is a member of Apocynaceae family. Leaves from *Nerium oleander* were shown to contain 0.018 to 0.425% oleandrin (weight/wet weight). Oleander extracts have been used for the treatment of indigestion, malaria, leprosy, mental or venereal diseases but the unconscious usage may cause toxicity.

Blood and urine sample on admission was assayed for oleandrin, the major cardiac glycoside of *N. oleander,* which has a wide geographical range and ecological distribution throughout the world and also in Turkey. Both specimens were extracted with ethylacetate: n-heptan (1:1) solvent mixture at 9.5 pH. Additionally, some parts of the oleander plant such as one flower, two leaves and one bark were chosen for extraction. These parts were cut and crushed in a 50 mL flacon to obtain about 2 mL sticky juice and then this was diluted with 3 mL water and extracted with the same solvent mixture.

All separated specimens were performed on a highly specific LC-MS procedure with gradient elution. Using this analytical setting, the average retention time for oleandrin was 0.9 min. The major ions monitored for oleandrin were m/z **577** and **433** indicating total molecular weight and without glycosides form, respectively. The highest sensitivity for this assay was obtained with 70 eV.

Qualitative results of the blood and urine samples on admission were compared with the plant extracts. Also qualitative result of the blood sample with urine sample was compared with each other. The most important thing was that the patient recovered without using any digoxin antibody such as Digifab or Digibind.

This procedure provided the toxicological data necessary to make diagnostic decisions about the patient when oleandrin was detected by toxicological screening. Also LC-MS appears to be the method of choice for the forensic-toxicological investigation of poisonings by cardiac glycosides.

Oleandrin, Poisoning, LC/MS

K10 Analysis of Hydrocodone, Hydromorphone, and Norhydrocodone in Urine Using Liquid Chromatography - Tandem Mass Spectrometry (LC/MS/MS)

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The goal of this presentation is to present a validated LC/MS/MS method for quantitative analysis of hydrocodone (HC) and metabolites and present data from human subjects administered HC.

The presentation will impact the forensic science community by providing data obtained from a method valdiation study of urinary HC and its metabolites.

Measurement of HC, a semi-synthetic opioid analgesic used for moderate and severe pain relief, can be used to monitor pain management compliance; however, HC levels can also be useful in drug testing cases to determine abuse or misuse of this commonly abused opioid. Hydrocodone is metabolized to its major metabolite, HM, and to a lesser extent to minor metabolites, NHC, and 6-α- and 6-β-hydroxymetabolites. Knowledge of metabolism and excretion profiles of administered HC can help in determining dose, time since last dose, and expected peak concentrations in subjects whose specific drug use is unknown. To effectively monitor and evaluate metabolism and excretion profiles, a sensitive and specific drug test is needed to ensure that the drug and its metabolites can be measured to the lowest detectable amount.

Standards spiked with concentrations of HC, HM, and NHC ranging from 1 - 10,000 ng/mL were prepared in opioid negative urine. Urine samples collected from subjects following HC administration were also evaluated. The LC gradient mobile phase consisted of (A) 0.1% formic acid and (B) acetonitrile; flow rate was set at 0.5 mL/minute. The internal standard solution contained 1µg/mL HC-D3, HM-D3 and NHC-D3 in methanol. A 250 µL aliquot of standard or urine was mixed with 25 µL of internal standard solution. Urine samples were hydrolyzed with β-glucuronidase, solid phase extraction (SPE) performed, followed by 10 µL injection on the LC/MS/MS system. The mass spectrometer was set in the ESI positive mode and analysis was performed using two multiple reaction monitoring (MRM) transitions per analyte. The MS/MS ion transitions monitored were *m/z* 300.2→199.1 and 300.2→171.0 for HC; *m/z* 286.1→185.0 and 286.1→157.0 for HM; *m/z* 286.2→199.1 and 286.2→241.1 for NHC; *m/z* 303.2→199.0 for HC-D3, 289.2→185.2 for HM-D3 and *m/z* 289.0→202.0 for NHC-D3.

The linear range was determined for this procedure by analysis on six different runs on concentrations ranging from 1 to 10,000 ng/mL of each analyte prepared in urine. The linear range was shown to be 5 to 10,000 ng/mL for HC and HM and 5 – 5,000 ng/mL for NHC with r value > 0.99 for all compounds. The limit of detection (LOD) was 2.5 ng/mL for HC and NHC and 5 ng/mL for HM. The limit of quantitation (LOQ) for all analytes in urine was 5 ng/mL. The method yielded good precision with RSDs of < 10% at 100 ng/mL HC, HM, and NHC. Based on this procedure, measurable amounts of HC, HM, and NHC were detected in human urine for up to at least 9 hours post dose HC.

The present study will provide a validated LC/MS/MS method for quantitation of HC, HM and NHC in urine and will also provide evaluation of urine samples obtained from individuals administered HC.

Hydrocodone, Metabolism, LC/MS/MS

K11 Determination of Titanium Element in Gingival Biopsies of Patients Treated With Dental Implants by Laser Ablation – Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS)

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After attending this presentation, attendees will understand the transition of the titanium element into gingival biopsies which determined using Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS).

This presentation will impact the forensic science community by determining low amount of biopsy materials for any titanium element with suitable Laser Ablation method equipped by ICP-MS.

Introduction: Titanium element is widely used material as an implant in medical applications especially in dentistry. The use of dental implants in the treatment of partial and complete edentulism has become a successful treatment modality in modern dentistry. Dental implants and their prosthetic parts are made of biocompatible materials. Today titanium and its alloys are the first choice to fabricate implant materials. Although titanium is a very inert material, it may corrode when in contact with the oral cavity. If titanium corrodes it releases ions which can cause local reactions such as pain and swelling or activate immune response.

Materials and Methods: The study was carried out in the Clinic of the Department of Oral Implantology at the Faculty of Dentistry and Institute of Forensic Science, Forensic Toxicology Laboratory in Istanbul University. The study group comprised 20 two-staged dental implants. Osteotomy and implant installation were performed according to the manufacturer's surgical protocol. The implants were exposed (second stage surgery) after three months and gingival biopsies were collected at each site. The biopsies were stored at -18°C until use. Samples were fixed to a lamina by an adhesive and dried in an oven at 90°C for 2 hours.

For comparison and prediction the change of elemental composition of gum tissues, sheep gum was used as a control matrix and confirmed that the sheep gum had no titanium element. An adhesive material fixed to a lamina with no sample was also used as blank for samples. Certified Standard Material (CRM), NIST 612 glass matrix was used for quality control sample. All samples fixed to lamina were analyzed by LA-ICP-MS. Titanium element was detected and compared with sheep gum and also with blank lamina.

Results and Discussion: Sheep gums were repeated five times and the mean value was accepted as the lowest amount for Titanium element. According to the results, some of samples showed titanium element significantly more than sheep samples. NIST 612 glass matrix showed that LA-ICP-MS system analyzed the titanium element close to certified amount. Moreover, there was no response to titanium in blank lamina which had no tissue. It can be concluded that adhesive didn't contain any contamination for titanium, and this may be suitable sample preparation process for biological tissues when they are studied using Laser Ablation.

Conclusion: Although all patients were exposed to titanium implant for three months, elemental quantitative results were variable. The best way to determine these kinds of patients might be monitoring

for their titanium level in biological samples such as urine or blood with specific time periods. Even if titanium is an inert material, these implants will be in contact with the oral cavity for a long time and may have toxic reactions in the body during the man's life. So, further studies will elucidate whether patients are under risk for titanium toxicity or not in time. Since gingival biopsy materials have very low amount, Laser Ablation may be the best method to determine the inorganic profile by ablating the tissue surface.

Dental Implant, Titanium, Laser Ablation

K12 Evaluation of Enzymatic Hydrolysis Efficiency for Buprenorphine Analysis

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After attending this presentation, attendees will understand how to apply Ultra performance liquid chromatography tandem mass spectrometry (UPLC/MS/MS) to buprenorphine analysis and evaluate efficiency of three different recommended buprenorphine hydrolysis methods using authentic samples.

This presentation will impact the forensic science community by providing an alternative, accurate method for buprenorphine analysis, and if UPLC/MS/MS analysis is not available, it offers an acceptable hydrolysis method.

Buprenorphine is quickly becoming a commonly prescribed analgesic for pain management. It is metabolized to norbuprenorphine, buprenorphine glucuronide, and norbuprenorphine glucuronide and is extensively eliminated in conjugated form. The glucuronides are cleaved by hydrolysis for conventional buprenorphine analysis by gas chromatography/mass spectrometry (GC/MS). Ultra performance liquid chromatography tandem mass spectrometry (UPLC/MS/MS) is an advanced technology that can measure intact glucuronides and allows reliable analysis of un-hydrolyzed buprenorphine samples. UPLC/MS/MS application can drastically reduce the cost and time associated with buprenorphine sample preparation. This study used UPLC/MS/MS to assess a non-hydrolysis method and compare the efficiency of the three most published buprenorphine hydrolysis methods.

Authentic buprenorphine positive samples (n=100) were separately analyzed by UPLC/MS/MS after sample pretreatment by dilution only or enzymatic hydrolysis using β-glucuronidase from *Helix pomatia (H. pomatia),* glusulase *or Escherichia coli (E coli). H. pomatia*-treated samples were incubated for four hours at 60°C. Glusulase-treated samples required one hour incubation at 60°C. The *E.coli* –treated samples were incubated at 37°C for two hours and sixteen hours for hydrolysis of buprenorphine glucuronide and norbuprenorphine glucuronide, respectively. Un-hydrolyzed samples were diluted only and then analyzed and used as references for hydrolysis efficiency. The UPLC/MS/MS gradient method for buprenorphine, norbuprenorphine, buprenorphine glucuronide, and norbuprenorphine glucuronide was previously validated with a linear range of 5-5000 ng/mL, precision < 6%, and coefficient of variation and accuracy ±18% of the target concentrations for all analytes.

The mean relative abundances of unconjugated buprenorphine, buprenorphine glucuronide, unconjugated norbuprenorphine, and norbuprenorphine glucuronide in the un-hydrolyzed urine samples were 0.2%, 19.2%, 8.6%, and 72.1%, respectively. This ratio is comparable with previously published distributions in plasma.[1] *H. pomatia* demonstrated excellent mean hydrolysis efficiency for both buprenorphine glucuronide and norbuprenorphine glucuronide (99.6% and 99.0%, respectively). Glusulase demonstrated very good mean hydrolysis efficiency for both buprenorphine glucuronide and norbuprenorphine glucuronide (97.3% and 95.4%, respectively). *E. coli* demonstrated satisfactory overall mean hydrolysis efficiency; giving 99.1% hydrolysis for buprenorphine glucuronide and 85.9% hydrolysis for norbuprenorphine glucuronide. There was a noticeable decrease in the total buprenorphine and norbuprenorphine concentrations of the hydrolyzed samples compared to the un-hydrolyzed samples suggesting possible degradation during hydrolysis. Statistical analysis was performed on the data for the hydrolyzed and un-hydrolyzed samples at a 95% confidence interval. The percent loss for buprenorphine and norbuprenorphine in the *H. pomatia* samples was 13.33% and 11.65%, respectively, and was statistically significant for both analytes (P=0.0055, P=0.0208). The percent loss in the Glusulase samples was 23.12% and 21.97% for buprenorphine and norbuprenorphine, respectively and was highly significant (P=0.0001, P=0.0007). Percent loss was 10.75% for buprenorphine and 24.79% for norbuprenorphine in the *E.Coli* samples. The decrease was significant for buprenorphine (P=0.0158) and highly significant for norbuprenorphine (P=0.0001).

UPLC/MS/MS can be employed for buprenorphine analysis and its application eliminates the need for sample hydrolysis because both the conjugated and unconjugated forms of buprenorphine can be detected and quantified. It also improves accuracy by precluding sample degradation due to hydrolysis. However, if the mode of buprenorphine analysis requires hydrolysis then *H. pomatia* is recommended as the hydrolyzing agent because it is most efficient and results in less degradation compared to the other two methods.

Reference:

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Buprenorphine, UPLC/MS/MS, Hydrolysis

K13 Automated and Comprehensive Analysis of Drugs in Whole Blood Using Cleanup Tips and LC/MS/MS

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The goal of this presentation is to present the development of an automated sample preparation method for the analysis of drugs in whole blood using minimal manual labor. The method is comprehensive and combines protein precipitation and "cleanup" for the analysis of acidic, basic, and neutral drugs.

This presentation will impact the forensic science community by demonstrating how automated sample preparation allows forensic labs to improve throughput, minimize sample handling, and increase confidence of results.

Recently, protein precipitated blood specimens have been analyzed directly by LC/MS/MS without additional solid-phase extraction (SPE) or cleanup procedures. In this study a comparison of samples analyzed with and without cleanup is shown. The advantages of using a cleanup procedure are: (1) the method is rapid because it does not involve condition, wash and elution steps; (2) less maintenance issues are required for the LC and MS instrumentation; (3) better sensitivity due to elimination of ion suppression and matrix effects; (4) better reproducibility for qualitative and quantitative measurements; and (5) more confidence in the screening and confirmation of drugs and metabolites.

A cleanup tip was developed that is used to simultaneously filter the proteins precipitated from whole blood and extract the sample matrix components. The extractions are performed completely automated using a dual rail GERSTEL MPS-2 instrument interfaced to an AB SCIEX 3200 Q-Trap instrument. The automation allows the analysis to be non-tedious and improves sample integrity by minimizing manual sample handling. Use of the Q-Trap permits the ability to obtain full scan mass spectral data of drugs and metabolites, even at low concentrations. The full scan capabilities gives greater confidence in compound identification, and is a great resource for unknown screening that is common in forensic toxicology.

In this study, analyses of over 60 drugs and metabolites in whole blood are shown using the cleanup tips. The drugs and metabolites include opiates, opioids, benzodiazepines, analgesics, anticonvulsants, stimulants, and hallucinogens. Recoveries are greater than 70% and RSDs less than 10%, with most recoveries being approximately 90%. Direct comparisons are shown of samples treated with and without cleanup.

Sample Preparation, LC/MS/MS, Automation

K14 Analytical Methods Development for Identifying and Quantifying Synthetic Cannabinoid Substances

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After attending this presentation, attendees will learn about the details of analytical methods including gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and infrared spectrometry (IR) for analyzing synthetic cannabinoid products containing the JWH-018 ingredient. The chemical characteristics of the K2 and K3 products along with the metabolic breakdown of the active compounds in the urine matrix will be discussed. The findings and methodologies will contribute to future development of a protocol for analyzing banned synthetic cannabinoid substances.

This presentation will be impact the forensic science community, as well as, the drug enforcement community by providing important information on the development of laboratory methods for analyzing synthetic cannabinoid substances that are different from natural marihuana products. The new laws banning these substances will require robust methods based on GC-MS, LC-MS, and IR spectrometry for characterizing products seized in police raids and assessing the levels of the metabolites in the urine samples of suspected users.

Recently, several states have outlawed the sale, use, and possession of synthetic cannabinoids. For instance, Tennessee has banned the sale of synthetic cannabinoids (more specifically JWH-018, JWH-073, HU-210, and HU-211) as of July 1, 2010. The popularity of these drugs is largely due to the lack of an analytical protocol for analyzing the synthetic cannabinoid substances for enforcement purposes. The use of these illegal drugs is often associated with trying to get "high" or intoxicated while avoiding the detection of the metabolites of these synthetic cannabinoids in order to pass the standard drug test for natural cannabinoids. The development of an analytical method for determining the characteristic metabolites of synthetic cannabinoids will provide a reliable means of identifying individuals using synthetic cannabinoids and studying the detection periods of these metabolites in urine samples. These drugs are commonly available under the name K2 or K3, each of which have the active ingredient of JWH-018.

Since the emergence of synthetic cannabinoids and their "herbal blends" in the markets, little research has been published on the urine metabolites of these products. Some synthetic cannabinoids are extremely potent and regulated by the U.S. Drug Enforcement Agency (DEA). For example, HU-210 is 100 times more potent than the active ingredient in marijuana, Δ9-tetrahydrocannabinol (THC), and is subsequently considered a Schedule I controlled substance.[1] Analysis of the current commercially available synthetic cannabinoids has shown the presence of JWH-018 and CP 47,497-C8, as well as the derivatives of these two compounds.[2] The allure of these compounds is that they act on the same receptors (CB1 and CB2) resulting in a "high" without triggering a positive drug test for marijuana use.[3] The most recent publication related to the metabolites of synthetic cannabinoids focused on the analysis of urine samples following the administration of "Tropical Synergy" obtained from Russian law enforcement. The study confirmed the presence of two synthetic cannabinoids, JWH-018 and CP47,497-C8. Following urinalysis via gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS), several hydroxylated derivatives of JWH-018 and CP47,497-C8 were found.[4]

In this study, the techniques of GC-MS, LC-MS, and infrared spectrometry (IR) are used to analyze synthetic cannabinoid compounds present in the herbal products, smokes, and urine samples of test subjects. Attenuated total reflectance and long path-length gas cells are used with IR spectrometry for both herbal product and smoke analysis. Solid phase extraction as well as gas sampling bags and sorbent tubes are used for the mass spectrometric analysis of cannabinoid constituents. Herbal mixtures with synthetic cannabinoids differ from manufacturer to manufacturer and the components of their mixtures are rarely given in the package labels. The combined GC-MS, LC-MS, and IR analysis provide unambiguous identification of constituents in the K2 and K3 products. The development of methods for characterizing the commercially available herbal products, the constituents in the smoke and smoke residues, and their metabolites in the urine matrix would be a significant step for the enforcement of laws regarding these illegal synthetic cannabinoid substances.

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Illegal Drugs, Synthetic Cannabinoids, Marijuana

K15 Stability and Reproducibility Studies for Carbohydrate Deficient Transferrin Analysis Using Capillary Electrophoresis

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After attending this presentation, attendees will develop an understanding of the protein Transferrin, and gain an understanding as to its stability and reproducibility and therefore its credibility as an analytical biological marker.

This presentation will impact the forensic science community by introducing a technique that, because of its stability and reproducibility,

can be used in routine toxicological analysis concerning questions of chronic alcohol abuse.

The present research addresses the quantitative analysis of Carbohydrate Deficient Transferrin (CDT) levels in biological samples using capillary electrophoresis for reproducibility and repeatability as well as the analyte's stability *in vitro*.

Transferrin is a glycoprotein responsible for binding iron and transporting it via blood throughout the body. Multiple transferrin isoforms have been observed based on the presence of oligosaccharide chains containing acetylglucosamine, galactose, mannose and sialic acid. The sialic acid residues are in terminal positions of these chains and are the only part of the chain with a negative charge. The number of sialic residues in a transferrin molecule expresses the degree of transferrin glycosylation in an individual, which is usually:

Tetrasialio-transferrin: 75%
Pentasialio-transferrin: 15%
Trisialio-transferrin: 5%
Disialio-transferrin: 2%
Hexasialio-transferrin: 2%
A-, Mono-, Hepta-, Octa- sialo-transferrin: <1%

Carbohydrate Deficient Transferrin (CDT) refers to the sum of the disialic, monosialic, and asialic groups. Research has indicated that individuals with a pattern of consuming > 50-80 grams of alcohol (approximately > 5-8 drinks) a day for at least seven consecutive days will have an increased CDT value. This indicates sustained alcohol consumption and provides information about an individual's drinking habits (indicate a potential alcohol abuser).

One sensitive and specific instrumental method used to detect CDT is Capillary Electrophoresis (CE). CE technology employs a voltage potential to a narrow-bore silica capillary and separates components based on size and charge. CE technology can separate the different transferrin glycoforms and, by assessing peak area ratios, determine the percentage of CDT in human serum.

The control and one sample serum were run six different days, six injections per day to examine both the intra-day variability (repeatability) as well as the inter-day variability (reproducibility).

Three different storage conditions were utilized to examine the % CDT values and assess the stability of CDT in serum. Aliquot sets from four different sample sera were stored on a lab bench top at room temperature (25°C) over a nine-week period, in the refrigerator (approximately 4°C) over a ten-week period, and in the freezer (approximately -20°C) over a seventeen-week period. The % CDT was also checked every two weeks. Each serum sample along with the control was injected twice. The sample aliquots were stored in the freezer and analyzed every two weeks over an eight-week period.

The data generated during these studies indicated that CDT remains stable for extended periods of time when stored under various conditions but will remain stable the longest when stored at either 4°C or -20°C. Even if other studies are required to check the stability of the CDT related glycoproteins in serum samples over a longer span of time, the assessment of CDT under standard laboratory conditions highly supported the adoption of CDT as an indicator of alcohol abuse in the clinical and forensic environments.

CE technology proved again to be a simple and automated analytical tool producing easy reproducible and repeatable determinations of CDT in human serum, suitable for application in the daily routine of a toxicology laboratory.

Alcohol Abuse, Carbohydrate Deficient Transferrin, Capillary Electrophoresis

K16 Amitriptyline and Morphine Determination in Larvae of *Lucilia Sericata* and Decomposed Liver Using LC-MS/MS

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After attending this presentation, attendees will understand the reliability of insect larvae as samples for toxicological investigations and the methods that were developed in analysis of drugs in larvae and liver samples.

This presentation will impact the forensic science community by providing the real toxicological evidence from corpse and larvae.

Analytical entomotoxicology is a basic new of forensic toxicology, where a few studies exist in literature. The goal of this study is to evaluate the use of insects as alternative specimens for toxicological evidence. For this purpose, larvae of *Lucilia sericata* were reared on samples of minced chicken liver treated with different concentrations of amitriptyline and morphine; regarding therapeutic, toxic, and potentially lethal doses. A method was developed for amitryptiline determination in larvae and liver and morphine detection in larvae. Amitryptiline and morphine was detected in all tested larvae samples, confirming the reliability of these specimens for qualitative toxicology analysis. Quantitative concentrations of amitriptyline measured in larvae were correlated with levels in liver tissue. The recoveries for morphine was not repeatable and the method could only be used to detect this drug qualitatively. These observations bring new elements regarding the potential use of drug analysis in larvae for estimation of drug levels in human tissues.

Introduction: Insect colonization patterns are the most common factors utilized for postmortem interval (PMI) estimation, especially when the discovery of the corpse is delayed and the soft tissues are decomposed. Diptera larvae feed on decomposed tissues containing chemical substances because of antemortem drug exposure. Because of this, the use of necrophagous insect specimens can be valuable as evidence for qualitative drug detection and sometimes quantitative drug determination, when the liver is almost completely decayed.

Materials and Methods: ***Breeding*** - Approximately 400 eggs of *Lucilia sericata* which is a common necrophage species of Diptera in Europe, were deposited on different concentrations of amitriptyline with homogenized tissues of chicken liver (250.0 g). Each chicken liver homogenate was spiked with different amitriptyline concentrations. C_1, C_2, C_3, C_4 were 500.00, 3000.00, 7000.00, 10000.00 ng/g, respectively. The non-spiked blank liver was regarded as C_0. ***Sampling*** - At the end of the 117(±0.5) hours period (beginning from the egg phase to third feeding larvae phase), the larvae were collected from each liver, along with the corresponding liver sample for analysis. ***Extraction procedure*** - Approximately 0.500 g larvae and liver were homogenized in a 0.9% NaCl solution and an original LLE extraction procedure is developed. Organic layer of each sample was evaporated to dryness under N_2, reconstituted in methanol and analyzed by ESI LC-MS/MS.

Results and Discussion: Amitriptyline and morphine were analyzed in this study. MRM method was developed with determined R*t* and selected m/z values. The ions 286.1→ 201.1 for morphine and 278.0→ 91.0 for amitryptiline were monitored. The method was validated in terms of specificity, linearity, accuracy (recovery ≥82%), and precision and LOD and LOQ values were determined using

Eurachem method. Validation results established that routine quantitative amitriptyline and qualitative morphine determination can be achieved in liver and larvae matrices. Also at this study; a formula was suggested for a back-calculation from the results that were obtained from the decomposed liver matrix and larvae collected at the end of the 117(±0.5) hours period, to the 0th hour liver concentration.

Entomotoxicology, Tricyclic Antidepressants, Opiates

K17 An Application of Speciated Isotope Dilution Mass Spectrometry (SIDMS) for Simultaneous Drug Quantitation of Gamma-Hydroxybutyric Acid (GHB) and Gamma-Butyrolactone (GBL) in Urine and Blood Matrices

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After attending this presentation, attendees will learn how Speciated Isotope Dilution Mass Spectrometry (SIDMS) can be applied to the forensic community. This presentation will provide an example of GHB and GBL to show how this method can be applied.

This presentation will impact the forensic science community by showing the community a quicker and more accurate way to quantitate drugs with the example of GHB and GBL.

There are currently two major problems in the forensic science community: scrutiny of analytical methods and a rapidly growing backlog of samples. An accurate, rapid and simultaneous measurement of GHB and GBL in urine and blood was developed to combat these issues. This legally defensible method for analyzing both *gamma*-hydroxybutyric acid (GHB) and *gamma*-butyrolactone (GBL) simultaneously uses speciated isotope dilution mass spectrometry (SIDMS). Current methods use gas chromatography mass spectrometry (GC/MS) and are not able to quantitate both GHB and GBL simultaneously; therefore, multiple extractions are required in order to quantitatively analyze GHB and GBL. To perform SIDMS, deuterium labeled GHB and carbon labeled GBL were utilized to spike the samples for quantitation. Once the naturally occurring analyte is spiked with the isotopically enriched analyte, SIDMS can account for any inter-conversion that occurs between GHB and GBL during sample preparation or analysis. After spiking the samples, a mixed-mode (phenyl and propyl sulfonic acid) solid phase extraction column was used for the filtration extraction of GHB and GBL from urine and blood samples. Mass spectrometry studies were done using electrospray ionization. Method validation was completed with triplicate sample preparation and analyses (n =9) with a known concentration of GHB and GBL in standardized urine and blood. Significant values of GHB and GBL were chosen based on previous studies completed in the literature. Concentration values of 5 ppm, 10 ppm, 200 ppm, and 400 ppm were used. Endogenous levels of GHB average below 10 ppm. Some studies have reported endogenous cutoff levels of GHB should be 6 ppm in urine to avoid false negatives. GHB overdoses were reported at an average of 200 ppm and have been seen as high as 400 ppm. The experimental values and the standard values were in agreement with the 95% confidence interval. By using SIDMS, inter-conversions between GHB and GBL can be accounted for and the correct quantification of both analytes can be made. Temperature and pH levels were varied to stimulate conversion between the two analytes, GHB and GBL. The inter-conversion was accounted for in the SIDMS calculation, which demonstrates the benefit for the use of this method in the forensic science community. Calculations were made to account for the inter-conversion, which demonstrate the use of the SIDMS method for drug quantitation. This method can help forensic scientists by providing a procedure that is legally defensible and quicker than other traditional methods of analyzing GHB and GBL. This method can be beneficial to the forensic science community.

Quantitation, SIDMS, GHB

K18 Validating Immunoassay ELISA Kits to Detect Eighteen Benzodiazepines at Low Levels

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After attending the presentation, attendees will understand the process of validating an immunoassay benzodiazepine ELISA kit for low concentration of drugs.

This presentation will impact the forensic science community by demonstrating how changing the experimental parameters for an ELISA kit will allow you to detect more drugs at needed concentrations.

Reports have shown that 30-40% of drivers take benzodiazepines and that the use of these drugs could have impairing effects. The Orange County Crime Lab (OCCL) recently validated an immunoassay benzodiazepine ELISA screen to detect the 22 benzodiazepines confirmed by an LC/MS/MS method at similar detection levels. The main benzodiazepines prevalent in casework are alprazolam, diazepam, lorazepam, and clonazepam and detection limits of 2, 10, 4, and 3 ng/mL, respectively are required. The validation process addressed limits of detection, blanks, sample volumes, possible interferences, and saturation curves for all detected benzodiazepines. The validation determined that Temazepam at 3 ng/mL is the best benzodiazepine to use for the limit of detection, and allows the OCCL to detect 18 of the 22 benzodiazepines seen via LC/MS/MS. Only four benzodiazepine metabolites yield false negative results when no other benzodiazepines are present.

ELISA Validation, Benzodiazepines, Low Concentrations

K19 Detection of Acute Diazinon Exposure in Postmortem Bone Samples

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After attending this presentation, attendees will understand the techniques of analyzing the acute diazinon exposure in postmortem bone samples in explaining the mechanism and cause of death.

This presentation will impact the forensic science community by the mechanism of diazinon exposure in understanding the cause of death and the importance of detecting it with the forensic toxicology lab techniques.

Forensic toxicological analyses have traditionally focused on the use of blood, body fluids, and certain organs in examinations of deaths due to intoxication. However, in some situations, putrefaction and contamination make proper sampling from tissues and blood impossible, such as in exceedingly degraded exhumation cases. In these cases, bone might be useful as an alternative specimen since it is a potential depot for pesticides and other chemical agents.

This third study is focused on the use of alternative specimens where putrefaction and contamination make proper sampling from

tissues and blood impossible. The first study, regarding this issue, was the use of bone marrow in detection of Endosulfan and Diazinon. The second study dealt with use of adipose tissue in detecting chronic organochlorine exposure. As the following experimental research after the study by Akcan et al. in 2009 and Daglioglu et al. in 2010, the present study separately deals with the use of bone samples in detection of diazinon in a longer postmortem period. In order to find out the value of use of alternative biological samples in long period of postmortem cases, further series of experimental researches examining different alternative samples are currently designed.

Diazinon is widely applied to control agricultural pests in the Cukurova region which is the largest agricultural area in Turkey. In this region, diazinon takes place as the most common cause for organophosphate related intoxications. Most poisonings by diazinon are due to suicidal or accidental exposure and usually occurs by oral ingestion. Therefore, detection of diazinon in postmortem cases or putrefied corpses is of high importance in forensic toxicological analyses.

The goal of this study is to determine diazinon in bone samples of close term postmortem cases and putrefied corpses of pesticide treated rabbits, in order to show and emphasize the value of boney tissue, a potential depot for most chemical agents, as an alternative toxicological sample of long term after death. A 2500 mg/kg dose of diazinon was orally given to six rabbits through a gavages tool. One rabbit was not treated with anything and served as a blank control sample. The rabbits were buried in soil, after obtaining postmortem right femoral bones of each as first sample. All seven rabbits were exhumed three months later, and remaining left femoral bones were sampled. The bone specimens were cleaned of any overlying muscle and putrefied tissue using a scalpel. The samples were subsequently rinsed with deionized water until the wash was clear and free of debris and air-dried. The bone was weighed (2 g), cut into slivers, soaked in methanol and rotated for 16 hours. Solid-phase extraction (SPE) and gas chromatography/mass spectrometry (GC/MS) were used for the analysis of diazinon in bone samples. The methanol supernatant was removed and then loaded into sample extraction cartridge, the eluent was evaporated to dryness under a nitrogen stream, reconstituted with methanol, and then analyzed by GC/MS. Ethion was used as an internal standard. Limit of detection (LOD) for diazinon was 0.03 mg/kg and limit of quantification (LOQ) for diazinon was 0.10 mg/kg. Calibration curve was prepared with seven sample concentrations and correlation coefficient were $(r) > 0.999$, the values obtained for intra- and interday precision and accuracy were within the criteria usually accepted for bioanalytical method validation.

The mean concentrations of diazinon in bone taken just after death and bone samples of exhumed corpses were 11.52 and 7.97 mg/kg, respectively.

These results suggest that in pesticide intoxication related deaths when other specimens are unavailable due to degradation, bone samples should be considered as useful alternative specimen.

Diazinon, Bone, Forensic Toxicology

K20 An Overview of Modern Chromatographic Methods for Analysis of Anesthetics

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After attending this presentation, attendees will be aware of the past and current trends of chromatographic analysis of general and local anesthetics, and understand the history of instrumental chromatographic analysis of anesthetics goes back to the 1950's, when the first anesthetic lidocaine was analyzed. The technique of chromatographic analysis has taken over all other methods of analysis due to their rapidity and ease of sample preparation.

This presentation will impact the forensic science community by making forensic experts aware of what technique, pre-sampling requirements, and extraction methods should be used for the analysis of anesthetics. Also, the presence of modern detectors and capillary columns has enabled the difficult analytical part easier for the forensic analysts.

Several attempts have been made to analyze various classes and combinations of anesthetics by spectroscopic, electrophoretic, and chromatographic methods. Chromatographic methods have taken over other conventional methods for their high detection limits, high resolution, sample recovery, and no prerequisites of sample pre-analysis. This review focuses on the development of the chromatographic methods which includes the pre-analytical aspects such as extraction from pharmaceutical samples, body fluids such as blood, serum, plasma, CSF, hair, viscera, etc., selection of appropriate chromatographic technique, and comparison of the output after analysis. The various parameters which judge the ambiguousness of a particular technique for a said drug were LOD, LOQ, RSD, and mean recovery. The three major chromatographic analytical methods for detection of trace amount of about 15 anesthetics from various sources are reviewed. In addition to this, methods for analysis of various anesthetic combinations are summarized. This review describes various developments taken place during the last twenty years on applications of chromatographic techniques in clinical measurement of various anesthetics.

Chromatographic Methods, Anesthetics, HPLC

K21 Determination of Lignocaine Hydrochloride and Bupivacaine Hydrochloride in Pharmaceutical Samples Using Thermogravimetry, IR Spectrophotometry and Atomic Absorption Spectrophotometry

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After attending this presentation, attendees will understand the analysis and shortcomings of the anesthetics analysis countered as exhibits in case of anesthesia overdose. The short half-life and rapid degradation of these drugs have puzzled analysts for a fairly long period. Hence there was a need of an analytical method which would ensure the presence of drug in pharmaceutical samples, leftover vials, and such exhibits.

This presentation will impact the forensic science community by serving as a process aspect for a simple and rapid quantitative analysis of anesthetic drug overdose in cases where even qualitative analysis would have been difficult due to short half life of anesthetic drugs.

Ion associate complexes of lignocaine hydrochloride and bupivacaine hydrochloride with five metal tetrathiocynates (i.e., nickel, chromium, zinc, cobalt, manganese, and phosphomolybdate) were prepared. The precipitated ion associates were subjected to elemental analysis and further spectroscopic studies were done to determine the metal content and the association and stability of the metal-oxygen bond between the drug and the metal thiocynate. Solubilities of these solid ion associate complexes were studied and their solubility products were determined at different temperatures at the optimum pH for their quantitative precipitation. The thermodynamic parameters ΔH, ΔG, ΔS were calculated after the thermal studies for the dissolution of lidocaine and tetrathiocynates. The development of the AAS method was done by precipitating the drug in excess inorganic metal complex ions i.e.

tetrathiocynates and further determining the amount of excess metal ions unprecipitated by the drug by AAS. The method was applied for five metal tetrathiocynates and phosphomolybdate complexes in pure and pharmaceutical samples. The spectroscopic data revealed association of drug with metal with prominent M-O bonds indicating the stability of the drug metal complex. Further solubility and thermal studies revealed the physical and chemical characteristics of the complexes. The AAS studies of the complexes showed high precision and accuracy and gave a mean recovery of 99%. This research overcomes the shortcomings of analysis of anesthetics due to their short half life and minimal shelf life.

TG-DSC, AAS, Anesthetics

K22 Can Pharmacogenetic Studies Improve the Effectiveness of Methadone Maintenance Programs?

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After attending this presentation, attendees will better understand how the effectiveness of methadone maintenance program can be improved by pharmocogenetic studies.

This presentation will impact the forensic science community by explaining how the utilization of modern technologies, including polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and single nucleotide polymorphism (SNP) for studying genetic variants and gas chromatography-mass spectrometry (GC-MS) analysis of pharmacokinetic parameters, would help place the maintenance program on a higher scientific ground.

Following the implementation of the "harm reduction" policy, methadone (MTD) has now been widely adopted for "treating" heroin addicts in Taiwan. In humans, MTD is metabolized by *N*-demethylation to 2-ethylidene-1,5-di-methyl-3,3-diphenyl-pyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenyl- 1-pyrrolidine (EMDP). It has been reported that: (a) treatment effectiveness was highly affected by the prescribed dose; and (b) patients receiving the same dose responded differently. With this understanding, a study was conducted on pharmacogenetic parameters of patients in a local MTD maintenance program, focusing on understanding the relationships between patients' plasma level of MTD (and its metabolites) and their treatment dose and genetic polymorphisms of ABCB1 and CYP2C19. Gas chromatography-mass spectrometry was used for the determination of these analytes' concentrations in plasma, while polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and single nucleotide polymorphism (SNP) genotyping assay were used for the analysis of genetic variants. The concentration of MTD, EDDP, and EMDP found in 55 patients (prescribed dose = 10-165 mg/day) were 39.2-805 ng/ml, 1.18-127 ng/ml, and < 0.5-38.3 ng/ml, respectively. For the low-dose group (< 50 mg/day), correlations of MTD dosage and the observed plasma concentrations of MTD and EDDP were $R^2 = 0.638$ and $R^2 = 0.680$, respectively. For the high-dose group (≧ 50 mg/day), the corresponding correlations were $R^2 = 0.141$ and $R^2 = 0.103$. The latter finding suggests that the observed concentrations of MTD and EDDP might be associated with these patients' pharmacogenetic characteristics. Findings derived from PCR-RFLP and SNP genotyping assays include: (a) patients with GT, GA, TT, TA, and AA variants in their ABCB1 G2677T/A were associated with high EDDP plasma level ($p = 0.003$); (b) patients with 681A and 990T in CYP2C19 were associated with low EDDP plasma level ($p = 0.015, 0.010$, respectively); and (c) no definite pattern of plasma drug concentration could be established ($p > 0.05$) for patients with SNP C1236T and C3435T (in ABCB1) variants. In conclusion, understanding pharmacokinetic and pharmacogenetic parameters can potentially improve the effectiveness and safety in the implementation of the maintenance program.

Methadone Maintenance Program, EDDP, Pharmacogenetics

K23 Taking the High Road: A Look at San Diego Auto Accidents Involving Inhalant Abuse

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After attending this presentation, attendees will have a greater knowledge of the toxicology and methodology associated with hydrofluorocarbon analysis as well as the symptoms associated with inhalant abuse, as two case studies will be discussed.

This presentation will impact the forensic science community by illustrating the catastrophic effects of car accidents caused by inhalant abuse and by illustrating the importance of an inhalant method in the laboratory. Included in the discussion will be a look at inhalant abuse demographics with a portion focusing on the prevalence of inhalant abuse in the military. Method creation and validation will also be discussed.

Inhalants can be found everywhere; in schools, homes, offices, bedrooms, garages, and supermarkets. Inhalant abuse is considered the "intentional or deliberate inhalation of chemical vapors to achieve intoxication," and this can be done with any product that produces vapors. According to the U.S. Consumer Product Safety Commission, there are more than 1,000 products that contain dangerous solvents that can be abused. Inhalants are often seen as less harmful when compared to other recreational drugs but often "can result in total unconsciousness and even death the first, tenth, or one-hundredth time." The mechanism of inhalant deaths will be discussed along with the definition of sudden sniffing death and delayed death.

In San Diego City, approximately 62% of the drivers under the legal limit BAC of 0.08 grams % have something other than ethanol in their system; and some of these drivers' test results come back negative for commonly used recreational drugs. Due to the lack of apparent cause of the intoxication, the Forensic Chemistry Unit at the San Diego Police Department was asked to develop and test a method for presumptively identifying commonly abused inhalants (or volatile substances) in blood samples. The method created focuses on hydrofluorocarbons, namely 1,1-Difluoroethane and 1,1,1,2-Tetrafluoroethane, along with Ethylene Dichloride, and Toluene. A need for this kind of analysis became a priority after a 9-year-old girl was fatally killed in a car accident involving a driver who was under the influence of a volatile substance. This case will be discussed in depth during this presentation.

Inhalant intoxication is very similar to alcohol intoxication producing symptoms such as slurred speech, lack of coordination, euphoria, lightheadedness, delusions, and dizziness. Higher levels of intoxication can also produce confusion, nystagmus, and decreased reflexes. In the driving cases used in this study, drivers who were high on volatile substances exhibited many dangerous driving traits such as straddling the center lane, stopping without cause in a traffic lane, braking erratically, as well as accelerating and decelerating rapidly.

Manufacturers have implemented various techniques to deter the abuse of their products; this is referred to as product modification.

Product modification can be done in three ways, by removing the harmful component, by adding a deterrent, or by modifying the package so that it is less likely to be misused. Product modification in the United States, as well as in Australia, will be discussed.

Inhalant Abuse, Toxicology, Driving

K24 Alcohol Analysis in the 21st Century: Analysis, Reporting, and Interpretation

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After attending this presentation, attendees will gain knowledge about conducting thorough alcohol analyses in DUI cases, from analysis to accurate reporting, as well as, interpretation of the data using BAC software.

This presentation will impact the forensic science community by demonstrating a very thorough approach to the analysis of blood alcohol concentration (BAC) in DUI cases. In addition to the traditional approach of Gas Chromatography (GC)-Headspace analysis, uncertainty of the analysis will be introduced as well as the use of BAC software to extrapolate what the blood alcohol level was at the time of the incident in question, as in the case of an accident, based on the measured BAC. The application of this analysis method to several DUI cases will be discussed.

The analyses were performed on an GC-Headspace instrument that had been validated to confirm its accuracy based on results obtained from another instrument. A dual column system was used, the first of which quantitated the concentration of ethanol, while the second column served to confirm the presence of ethanol. The uncertainty of the measurements is taken into consideration by addressing a variety of areas, including the uncertainty in the purity of the standards used, the accuracy of the pipettes used, and the accuracy of the aliquoting of samples. Once the measurement was made, the BAC software was then used to extrapolate the BAC at the time of the incident. Using this software, a range of levels can be calculated by averaging BAC levels obtained by several published equations for the estimation of BAC.

One of the cases analyzed was that of a 50-year-old white female who was involved in a car accident. Her measured BAC was 0.19 g/dL at 3:30 a.m., whereas the accident she was involved in took place at 11:30 p.m. She stated that she had two martinis, the first one at 7:30 p.m. and the second at 10:00 p.m. Using the BAC software, it was determined that she would have had to have consumed 5.13 oz of 100% alcohol/volume during the time period that she stated that she had been drinking. Using the extrapolation function of the software, it was determined that the BAC at the time of the accident would have been 0.26 ± 0.03 g/dL.

In conclusion, this method provides a thorough approach to the determination of BAC levels in DUI cases, resulting in a range of measurements that analysts can feel confident about.

References:

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2. LeBeau, Marc A. "Uncertainty of Quantitative Measurements." Society of Forensic Toxicologists meeting. Austin, Texas. 2006

DUI, GC-Headspace, Extrapolation

K25 Development of an LC/MS/MS Method for the Analysis of Fatty Acids

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After attending this presentation, attendees will be introduced to a novel LC/MS/MS method that can be used in the analysis of fatty acids. This method was applied to the analysis of fish oil and other omega-3-supplements and should be applicable to the analysis of other samples as well.

This presentation will impact the forensic science community by supplying a method that can alleviate time and effort in the analysis of fatty acids.

A common method for the analysis of fatty acids utilizes Gas Chromatography/Mass Spectrometry. This process requires several time-consuming and complicated steps to prepare the sample. This process also includes working with hazardous chemicals in order to derivatize the fatty acids. The current research focused on the development of an LC/MS/MS method that can be utilized in the analysis of fatty acids. This method was applied to the analysis of fish oil and other omega-3-fatty acid supplements. This method includes using a Restek Ultra C8 (3µm, 50x2.1 mm) along with a 20x2.1 mm Ultra C8 Guard Cartridge, also from Restek. Solvent A of the mobile phase was 50 mM formic acid/ 2 mM ammonium formate and Solvent B was 95% acetonitrile/water containing 50 mM formic acid and 2 mM ammonium formate. The following gradient was used: 50% B for five minutes, 50 to 100% B in 28 minutes, and holding at 100% B for two minutes. Heptadecenoic Acid was used as the internal standard. The essential omega-3 acids, Docosahexaenoic Acid (DHA) and Eicosapentaenoic Acid (EPA) were quantitated in the supplements. The limits of detection for DHA and EPA were 0.49 and 0.33 ug/mL, respectively. The limits of quantitation for DHA and EPA were 1.12 and 1.11 ug/mL, respectively. The linear range for DHA was up to 5 mg/mL and the linear range for EPA was up to 2 mg/mL. Supplements were analyzed before and after a base hydrolysis step. The before samples were simply diluted with 70/30 acetonitrile/chloroform and injected. Base hydrolysis samples were extracted with chloroform and then diluted with acetonitrile, keeping with the 70/30 ratio. At least 24 different brands of omega-3 supplements were examined using this method.

Laws and regulations surrounding dietary supplements may not be firm enough to cover the safety and quantity of the ingredients included in these products. Due to increasing production and use of these omega-3 supplements, some monitoring of the composition and safety of these products is warranted. The newly developed LC/MS/MS method simplifies the procedures involved in the analysis of fatty acids and provides a less time consuming and less hazardous method that can be applied to dietary supplements such as fish oil but should be applicable to other samples as well.

LC/MS/MS, Dietary Supplements, Fatty Acids

K26 An Investigation Into the Cellular Cytotoxicity of Benzylpiperazine (BZP) and Its Derivatives

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After attending this presentation, attendees will have an understanding of the methods for testing drugs of abuse for *in-vitro* cytotoxicity. Attendees will also have acquired a knowledge of the toxicity of BZP and a number of its major impurities, as well as, be able to relate this to the clinical significance.

This presentation will impact the forensic science community by providing, for the first time, evidence of the toxicity of BZP and its impurities at a cellular level. This allows us to begin to elucidate the mechanism of toxicity and thereby the treatment for those poisoned by these drugs.

The market for clandestine designer drugs has been expanding exponentially over the last decade. Piperazines are a group of psycho-active stimulants including 1-benzylpiperazine (BZP), 1-(trifluoromethyl-phenyl)piperazine (TFMPP), 1-(chlorophenylpiperazine) (CPP) and 1-(methoxyphenyl)piperazine (MeOPP).[1] Of these BZP is the most commonly encountered derivative marketed as a "herbal high"/"legal high" with the street name A2. BZP was originally produced as an anti-helminthic agent for livestock in 1944.[2] Studies on rats have revealed that BZP exerts its effects by elevating levels of serotonin and dopamine by blocking the re-absorption of these at neurological synapses producing the positive psycho-active effect.[1]

Over the last two years, there have been an increasing number of clinical reports published concerning fatalities after ingestion of BZP alone or in combination with other psycho-active agents. BZP is now a controlled substance in many countries. It is a class D drug in New Zealand, class C drug in the United Kingdom, and it is controlled as a schedule 1 drug in the United States.[3] There is very little published in the public domain regarding the toxicity of these drugs at a cellular level. Although there are numerous reports on the renal toxicity and concerns over chronic abuse, the actual activity of the drug has not been studied in cells from sites of biological filtration within the body.

This study examines the effects of short term exposure on immortalized cells derived from the kidney (CAKI-2), the liver (HepG2) and fibroblasts (3T3) to BZP, it's precursor piperazine hexahydrate, and synthetic by-product 1,4-dibenzylpiperzine (DZP).

Cells were exposed to the drugs for 1 hour at concentrations ranging from 0.783mg/ml-3.13mg/ml, following which they were assessed morphologically for evidence of cell death, either programmed cell death (apoptosis), uncontrolled cell death (necrosis), or no effect at all. To assess for cell death, cells were then labeled with annexin V to evidence the presence of apoptosis and propidium iodide (PI) for evidence of general cell death. These samples were analyzed using a BD FACS Calibur Flow Cytometer. Results were expressed according to the degree of annexin V and PI labeling as the percentage of viable cells (Annexin V -/PI -) versus the percentage of non-viable cells (Annexin V +/PI +). This data was confirmed using fluorescence microscopy and immune-labeling of the annexin V *in- situ*.

DZP causes comparatively higher levels of cell death giving LD^{50} values of 2.25mg/kg (HepG2), 1.50mg/kg (CAKI-2) and 1.20mg/kg (3T3). Piperazine hexahydrate resulted in minimal cytotoxicity, being most potent in its activity against HepG2 with an LD^{50} of 1.50mg/kg. BZP was most cytotoxic producing an LD^{50} value of 1.1mg/kg (HepG2), 1.57mg/kg (CAKI-2) and 1.40 mg/kg (3T3). Further to this it is shown that HepG2 cells display a lower threshold for sensitivity to these drugs than CAKI-2 or 3T3.

These data provide clear evidence of the cellular cytotoxicity of BZP and DZP and its synthetic by-products at levels likely to occur following the ingestion of these drugs. Data also indicate that in general the liver, site of primary biological filtration, is most sensitive to the actions of these drugs. This supports the clinical evidence that BZP produces a very real threat of causing hepatic toxicity.

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BZP, Phenylpiperazine, Toxicity

K27 A Statistical Analysis of Urine:Blood Data, and Oxycodone Redistribution: A Simple Ratio Will Not Suffice

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After attending this presentation, attendees will understand concept of a meta-analysis and the importance of statistical analyses in general. Attendees will gain an appreciation of the mathematical and statistical implications of simple ratios and what they imply.

This presentation will impact the forensic science community by introducing the concept of a quantitative review of literature rather than a qualitative review, and the importance of a rigorous statistical and mathematical analysis.

Meta-analysis, odds ratios, confidence intervals, prediction intervals, tolerance intervals, and bounds are demonstrated and these concepts are applied to several alcohol data sets including both blood and first- and second-void urine data and another meta-analysis on oxycodone redistribution will be demonstrated. A meta-analysis is a mathematical summary of previously done studies that address the same research hypothesis. The first meta-analysis, usually credited to Karl Pearson, a noted statistician, was done in 1904 to overcome the problem of small sample sizes and their reduced statistical power. A prediction interval is used to predict a value from a calibration curve while a confidence interval is used to express the uncertainties in the parameter estimates. Tolerance intervals are meant to contain a proportion of the population with a specified probability. Subject-level data available from urine-alcohol studies in the medical literature was extracted. Using multivariate regression techniques, the limits for urine-alcohol levels were critically examined from a statistical viewpoint taking into account whether first- or second-void urine samples were used, the assay method, and whether the subject was alive or dead. Oxycodone redistribution and, again, the improper use of only a simple ratio was also reviewed. The difficulties of modeling such data via regression strategies were examined.

Based upon an analysis of the data and pharmacokinetic, mathematical, and statistical principles, this presentation will show why the use of only a simple ratio is incorrect and misleading. Using the methodology described, it is easy to see that the per se values some state legislatures have incorporated into their laws and statutes allow innocent people to be convicted of a crime. Also, simple ratios and/or linear

regressions will not always solve the data analysis problem, and more complicated models will be required. This will require collaboration with a statistician and other specialists. It is concluded that the levels of urine alcohol specified by some states are set statistically improperly. Experts must incorporate statistical methods to properly summarize their data and report error intervals.

Meta-Analysis, Alcohol, Oxycodone

K28 Development of a Web Accessible Cheminformatic Mass Spectral Database for Shared Utilization by Forensic Laboratories

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After attending this presentation, attendees will understand how to using a free web accessible library with multiple spectral methodologies will allow electronic searching and comparison of unknown spectra against verified reference spectra including nominal mass, accurate mass, NMR, and FTIR spectra which can be applied to forensic toxicology, drug chemistry, and trace evidence such as ink dyes and explosives.

This presentation will impact the forensic science community by providing a free, community driven web accessible mass spectral library cataloging a standardized collection of compounds of forensic interest analyzed by various spectral technologies, thus enabling a more definitive compound identification.

Introduction: Cheminformatic databases are used for searching unknown spectra against reference spectra and for retrieval of chemical data such as structural information. In addition to traditional mass spectra, NMR and FTIR are also included to improve compound identification in some databases. The forensic utility of these databases varied due to the existence of relevant compounds and spectral methods, data quality, accessibility, and ability to search against reference spectra. Forensic applications of these databases routinely contain spectra from traditional instrumentation, such as electron ionization (EI) mass spectrometers (MS) and do not allow for cross-searching of other spectral methods. Direct Analysis in Real Time (DART) is a novel ion source coupled to an accurate mass time-of-flight (AccuTOF™) mass analyzer DART has been primarily employed for controlled substances identification by forensic laboratories. Currently, there are no public databases that incorporate DART spectra, requiring laboratories to create in-house discipline specific library resulting in unnecessary duplicity. These in-house libraries are not readily accessible to the public.

Methods: Currently, RTI International in collaboration with Virginia Division of Forensic Sciences (VDFS) have begun the development and mass spectral population of a forensic cheminformatic database containing mass spectra, NMR and FTIR which will be Web-accessible and free for anyone with internet access. Users upload spectra through a Web portal to an editorial review board where selected, external "collaborator reviewers" evaluate the spectra based on established criteria. RTI staff, as the "database curator," also review the data and the reviewers' recommendations on whether the spectra should be accepted, rejected, or accepted with revisions. If all criteria are met the spectra is approved and moved into the cheminformatic database for public accessibility. Otherwise, the spectra are either rejected or the contributing user may be contacted to determine if better spectra can be submitted. This multi-step, interactive forensic practitioner review process with established criteria of acceptance will help maintain the validity and reliability of the spectra. Duplicity of a compound within the cheminformatic databases can be limited or eliminated as appropriate. Inclusion of DART spectra into the database required spectral evaluation and comparison by RTI and VDFS laboratories. Several commonly altered DART parameters were investigated to determine whether enough spectral dissimilarity existed to cause a false identification in the developed database. Collection of reference drug standards at RTI using the same instrumental parameters as VDFS evaluated the inter-laboratory reproducibility. A form has been developed to systematically document and evaluate spectra under varying DART conditions and instrument parameters thus allowing the assessment of their affects on DART spectra and the matching quality within the database.

Results: The current public database consists of 2,400 EI mass spectra previously collected and maintained by the American Academy of Forensic Sciences Toxicology Section MS Database Committee and 224 compound records each with one to four DART spectra at different CID voltages collected simultaneously using function switching at VDFS. It appears that function switching sacrifices sensitivity for more spectral detail. All compounds have been analyzed and parameters documented for optimization and acceptable ranges by VDFS. Currently, the same procedures are being finalized for DART analysis at RTI.

Conclusion: Forensic laboratories that use mass spectral, NMR and FTIR technologies for forensic toxicology, drug chemistry, and trace evidence such as ink dyes and explosives may find this new Web-accessible Cheminformatic database to be a highly reliable and valid tool for identifying unknown compounds of interest.

Cheminformatics, Mass Spectral Database, Collection Standardization

K29 Identification of Markers of JWH-018 and JWH-073 Use in Human Urine

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The goals of this presentation are to inform the audience of the current knowledge regarding the urinary metabolites of JWH-018 and JWH-073 and to outline screening and confirmation methods for identifying the use of these compounds.

This presentation will impact the forensic science community by providing up-to-date information on the urinary metabolites of JWH-018 and JWH-073.

Recently, synthetic cannabinoids have garnered media attention as a legal alternative to cannabis. Sold as constituents of "herbal incense" under a wide variety of names including Spice, Yucatan Fire, Smoke, Sence, K2, Skunk, Space, K2 Citron, and K2 Blonde these compounds such as HU-210, JWH-018, CP 47,497, JWH-073, JWH-250, and JWH-200 are mixed with plant material and smoked. These synthetic analytes have a varying degree of selectivity and affinity for cannabinoids (CB_1 and CB_2) receptors and thus have different therapeutic and abuse potentials. As the popularity of these drugs increases, there is a developing need for analytical methods to identify and quantify the parent compounds in the herbal incense products as well as in biological matrices. On-going research will help identify metabolites of these compounds which can be used as markers of use in humans.

New drugs offer a unique challenge to the forensic toxicology community. Without authentic standard material for the multiple metabolites innovative methods of identifying the use of these compounds must be explored. Due to the lipophilic nature of these analytes, the parent compound is not excreted in urine emphasizing the

important of quickly identifying the metabolites as markers of use. Urine was collected from participants who smoked incense containing JWH-018 and JWH-073. These specimens were used to identify urine metabolites of these two compounds based on literature reports and LC-TOF analysis. Based on the literature and in-house analysis, JWH-018 and JWH-073 undergo mono-, di- and tri-hydroxylation followed by glucuronidation. Qualitative screen and confirmation methods for identifying exposure to JWH-018 and JWH-073 were developed and validated based on the presence of these urinary metabolites.

Specimens were screened for the monohydroxy glucuronide metabolites. Solid phase extraction was used to clean and concentrate unhydrolyzed urine specimens and extracts were analyzed on an LC/MS/MS for the detection of monohydroxy-glucuronide metabolites. The instrument was operated in positive ionization mode employing atmospheric pressure chemical ionization. Separation was achieved using gradient elution on a C18 HPLC analytical column. Source fragmentation of JWH-073-monohydroxy-glucuronide and JWH-018-monohydroxy-glucuronide was employed and the transitions resulting from the loss of the glucuronide moiety were monitored. Further fragmentation was then induced in the collision cell and two transitions monitored for identification purposes. The confirmation method employed is based on the presence of multiple urinary metabolites. Urine specimens underwent enzymatic hydrolysis and a liquid-liquid extraction prior to analysis. LC-MS/MS with electrospray ionization was performed on an Applied Biosystems™ API5000 system. Multiple transitions were monitored for each analyte. The following table summarizes the monitored transitions for the screening and confirmation methods:

Screening Method		
	Source Fragmentation	Collision Cell Fragmentation
JWH-018-monohydroxyglucuronide	534→358	358→155 & 358→127
JWH-073-monohydroxyglucuronide	520→344	344→155 and 344→127
Confirmation Method		
	Precursor Ion	Product Ions
JWH-018-monohydroxy	358	155 127 284 186
JWH-018-dihydroxy*	376 374	214 171 155 127
JWH-018-trihydroxy	374	189 171
JWH-073-monohydroxy	344	155 127
JWH-073-dihydroxy*	362 360	200 171 155 127
JWH-073-trihydroxy	378	189 171

*Multiple dihydroxy metabolites identified

Synthetic Cannabinoids, JWH-018, JWH-073

K30 Toxicological Analysis of Synthetic Cannabinomimetic Spice Drugs

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After attending this presentation, attendees will be informed of an LC/MS/MS method for determining the concentration of "spice" drugs in forensic blood and urine specimens.

This presentation will benefit the forensic science community by providing a method for qualitatively and quantitatively detecting seven emerging indole cannabinoid drugs of abuse using liquid chromatography-tandem mass spectrometry (LC/MS/MS). The lack of methods available to analyze these drugs makes detection difficult in suspected cases of "spice." Therefore, as the prevalence of use increases, the need for validated detection methods becomes important.

Synthetic cannabinomimetic drugs have been studied primarily for their activity as CB_1 and CB_2 cannabinoid receptor agonists. Additionally, their strong binding affinity for CB_1 receptors has made these synthetic drugs potent marijuana alternatives which have become increasingly popular in recent years. Many of these drugs are sold as herbal incense under the name "spice" or more commonly "K2" in the United States. JWH-018 is the most commonly found drug in these herbal blends. The drugs analyzed in this study include JWH-015, JWH-018, JWH-019, JWH-073, JWH-200, JWH-250, and WIN55212-2.

"Spice" toxicity can present itself in conflicting psychological states such as nausea, excitability, sedation, and panic. Physiological changes can include sweating, tachycardia, dyspnea, and xerostomia. Numerous hospitalizations as well as a suicide have been following reported acute doses of "spice." Furthermore, long term effects include panic attacks, blurred vision, muscle spasms, and a case of diagnosed dependence syndrome.

Liquid-liquid extractions were used to extract the drugs from blood and urine. Drug standards were spiked into negative blood and urine specimens. Various extraction conditions were compared in order to optimize extraction efficiencies of the drugs in both blood and urine. In the end, pH10 sodium borate buffer and ethyl acetate provided the best extraction efficiency. LC/MS/MS was used to analyze the extracted drugs and develop a method to qualitatively and quantitatively identify the drugs. Prior to LC/MS/MS analysis, drug optimization on the instrument was performed in order to select the appropriate qualifier and quantifier ions for each drug as well as the fragmentor and collision voltages. To ensure optimal chromatography, diazepam-D5 was chosen as the internal standard after comparison with hydrocodone-D3 and fentanyl-D5. Methanol with 0.1% formic acid was chosen as the mobile phase as it allowed for adequate separation of the compounds of interest.

The method was validated using the laboratory's validation guidelines. A five-point calibration curve was developed from 1-250 ng/mL. Linear ranges were from 1-100 ng/mL for all drugs except JWH-200 and WIN55212-2 which maintained linearity from 1-250 ng/mL with R^2 greater than 0.995 for all drugs. To validate the method, two extractions were performed on separate days. Accuracy and precision were calculated at 10 ng/mL and 100 ng/mL using three replicates for each concentration. LOQ for all drugs was 1ng/mL.

This presentation provides a rapid, sensitive method for determining the presence and concentration of several indole-based cannabinomimetic drugs in blood. The combination of chromatographic separation and ion monitoring with LC/MS/MS allows for multiple drugs to be accurately detected. This method can prove useful with the increasing rate of synthetic cannabinomimetic drug use in the population.

Spice, Cannabinoid, LC/MS/MS

K31 Analysis and Stability Determination of Salvinorin A and B in Human Blood, Plasma, and Urine by Liquid Chromatography Tandem Mass Spectrometry

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After attending this presentation, attendees will be able to describe the origins of and effects associated with abuse of *Salvia divinorum*, optimum methods for its analysis by liquid chromatography/tandem mass spectrometry, and limitations on its analysis based on analyte stability.

This presentation will impact the forensic science community by identifying a novel analytical approach to detection of an emerging hallucinogenic drug of abuse.

Salvinorin-A is an hallucinogenic compound that has no approved medical use in the United States. It is a naturally occurring, non-nitrogenous kappa opioid receptor agonist, and is the active component of the plant, *Salvia divinorum*, belonging to the mint family. The leaves

of the plant are typically dried, crushed, and smoked for their dissociative hallucinogenic effect. Plant concentrate or extract is also commercially available. Salvia is a potent hallucinogen with effects distinct from LSD, mescaline, and other hallucinogens. An effective dose in humans is reportedly in the 200 to 1,000 microgram range when smoked. Salvinorin A and Salvinorin B have both been identified in the leaf and leaf extract; however, Salvinorin B is present in much smaller amounts. The Salvinorin A and Salvinorin B contents have been determined to be in the range of 3.2–5.0/0.10–0.17 mcg/mg in the dried leaf products, and 4.1–38.9/0.26–2.42 in the "concentrated extract" products.

After smoking Salvia, subjects experience rapid onset of an intense hallucinatory dissociative effect, during which they cannot speak or recognize their surroundings, lose psychomotor coordination and are highly impaired. Acute symptoms resolve within 8 to 12 minutes; however, longer term and residual effects have not been studied.

A validated a liquid chromatography/tandem mass spectrometry (LC/MS/MS) method was developed for the identification and quantitation of Salvinorin A and B in human blood, plasma, and urine.

Salvinorin A and B were extracted from biological matrices treated and preserved with sodium fluoride by a single step liquid/liquid extraction. Salvinorin A was analyzed under positive mode ESI-LC/MS/MS and Salvinorin B was analyzed under negative mode ESI LC/MS/MS (ABI 5000 Tandem Mass Spectrometer, Shimadzu SIL 20A, HPLC). Ions monitored for Salvinorin A and its internal standard Salvinorin A-d3 are: m/z 433/373; 436/373. Ions monitored for Salvinorin B and its internal standard are: m/z 389/313; 391/359. HPLC conditions included 2% methanol in water gradient, vs water, at 1mL/min, on a Phenomenex Luna C8(2) 150cm column.

The linear range for this assay was established as 1–40 ng/mL for whole blood, plasma and urine. Response was linear, and the LLOQ was established at 1 ng/mL for both analytes. LLOD was approximately 0.25ng/mL. Within-run precision at the LLOQ was 3.2 % for Salvinorin A and 2.5% for Salvinorin B. The within-run accuracy was determined as 100±5% for both Salvinorin A and B.

Following development, the assay was validated according to laboratory procedure including assessment of inter- and intra- batch precision and accuracy, storage, extraction and autosampler stability, freeze thaw stability, dilution integrity, and recovery.

The stability experiments indicated that Salvinorin A and B in unpreserved urine were stable for 28 days refrigerated and frozen, Salvinorin A was stable for less than nine days at room temperature. Both compounds were unstable in sodium fluoride/potassium oxalate preserved whole blood at room temperature and refrigerated, being undetectable after one day. Samples that were preserved with sodium fluoride and EDTA and frozen, were stable for at least 28 days.

Challenges resulting from limited stability and likely low concentrations in human subjects make this a challenging assay for medico-legal applications and require the use of LC/MS/MS techniques.

Salvia Divinorum, Salvinorin A, LC/MS/MS

K32 Quantitative Analysis of Salvinorin A: (Salvia) in Blood

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After attending this presentation, attendees will be familiar with a technique for the extraction and quantification of Salvinorin A from blood specimens using solid phase extraction (SPE) and gas chromatography/mass spectrometry (GC/MS).

This presentation will impact the forensic science community by providing a new procedure for both qualitative and quantitative determination of the potent hallucinogen, Salvinorin A, from whole blood samples using GC/MS.

Salvia divinorum is a naturally occurring herb found within the *Lamiaceae* (mint) family. Salvinorin A is the trans-neoclerodane diterpene contained within its leaves that produces the plant's psychotropic properties. The drug is a potent naturally occurring hallucinogen. The availability and psychotropic effects associated with Salvinorin A have led to an increase in its use within the past decade. Studies have shown a growing trend in the *Salvia divinorum*-related media and internet traffic, as well as the use of the drug in persons age 12 or older. Salvinorin A is listed on the United States Drug Enforcement Administration's Drugs and Chemicals of Concern List but is not currently scheduled under the Federal Controlled Substances Act. Many states and other countries have already scheduled the drug and some are currently in the process.

Despite growing concerns regarding the recreational use of *Salvia divinorum,* published scientific literature describing Salvinorin A identification in toxicological specimens is very limited. Liquid chromatography/mass spectrometry (LC/MS) and related techniques have been reported. The objective for this study was to develop and validate a method for qualitative and quantitative identification of Salvinorin A in whole blood using a technique that was universally available in forensic toxicology laboratories (i.e. GC/MS). The development of the procedure evaluated potential protein precipitants, wash solvents, and elution solvents. The assay involves protein precipitation with 0.2 M zinc sulfate/methanol (20/80, v/v), mixed-mode solid phase extraction, a double wash step using hexane followed by hexane/dichloromethane (90/10, (v/v) and dichloromethane/ethyl acetate (80/20, v/v) as the elution solvent. Testosterone-d3 was used as the internal standard, and quantification was performed in selective ion monitoring mode. The ions selected were m/z 432, <u>273</u>, and 94 for Salvinorin A and m/z <u>291</u>, 249, and 124 for testosterone-d3 (quantitation ions underlined). The total run time was 23 minutes and the retention times for Salvinorin A and testosterone-d3 were 10.655 and 5.479, respectively.

The optimized GC/MS assay was evaluated in terms of limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, analytical recovery, linearity, interference, and carryover. The LOD and LOQ for the assay were determined to be 2 ng/mL. Precision and accuracy were evaluated at 15 and 150 ng/mL in blood. Both intra- and inter-assay CVs were in the range 4.5 - 7.7%. The 95% confidence intervals (95% CI) at 50 and 1000 ng/mL were 55.8 ± 5.3 and 1059.2 ± 43.5 ng/mL, respectively. Accuracy determined over a range of concentrations was 87-104% and analytical recovery of Salvinorin A was 88%. Calibrations were linear at concentrations as high as 5,000 ng/mL (the highest concentration tested). Carryover was evident at 2,000 ng/mL but this greatly exceeds concentrations expected in blood samples of forensic interest, which are typically one hundred-fold lower. The interference study included 27 commonly encountered drugs of abuse in addition to other her structurally related Salvinorins and divinatorins. No interferences were present, either qualitatively or quantitatively. This presentation provides a reliable and effective method for the detection and analysis of salvinorin A in whole blood by GC/MS at low concentrations of forensic interest.

Salvinorin A, Gas Chromatography/Mass Spectrometry (GC/MS), Blood

K33 Detection of Various Performance Enhancing Substances in Specimens Collected From Race Horses in Illinois: A Five-Year Experience

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After attending this presentation, attendees will understand the principles of testing of equine athletes for illicit performance enhancing substances, allowed medications, the scale of equine doping, and the most frequently detected drugs and substances.

This presentation will impact the forensic science community by demonstrating the pattern of use of performance enhancing drugs and substances in horse racing.

The goal of this study was to compile all analytical findings from five-year period of time (2004-2009) to determine what substances are used most frequently and to study drug use trends in general.

The Association of Racing Commissioners International classified all drugs having a potential of impacting the outcome from the race in the Uniform Classification Guidelines for Foreign Substances. All drugs and substances are categorized in five classes from Class 1 having the greatest potential for performance enhancing to Class 5, the least. All Illinois Racing Board rules and regulations regarding medication and testing for drugs are published in the General Assembly's Illinois Administrative Code, Part 603 (Medication). The rule lists thresholds of allowed medications in blood (serum) such as phenylbutazone, furosemide, flunixin, ketoprofen, thresholds of selected medications in urine (e.g., isoxsuprine, DMSO, selected anabolic steroids), as well as O-desmethylpyrilamine (pyrilamine metabolite) and benzoyloecgonine (cocaine metabolite). There is a "zero tolerance" established for Class 1-3 drugs. If the Class 4 or 5 drugs are found in the specimen, the quantification is required to be reported.

Testing protocol for urine and blood samples collected post-race from winning horses and others collected for various reasons as determined by track personnel in Illinois includes preliminary screening, on 65+ ELISA plates. The laboratory also analyzes the specimens collected postmortem and special exhibits such as syringes, needles, neat drugs, etc., found on race courses. In some cases the instrumental screening is performed using triple quad or ion trap LC-MS (e.g. anabolic steroids) or GC-MS (DMSO). All presumptive positive samples were subsequently confirmed by GC-MS or LC-MS. The use of alkalinizing agents, such as sodium bicarbonate, commonly called "milkshaking," is revealed by measuring the total carbon dioxide (TCO_2) level in plasma.

During the five-year period of time (2004-2009) 91,808 specimens were analyzed (45,210 urine and 46,598 blood samples) collected post-race from the winning thoroughbred and harness horses at eight race tracks in Illinois. The total number of violations reported was 413 (0.45%). The total number of violations reported in blood was 207 (0.44% of all blood specimens), and in urine 206 (0.45% of all urine specimens). The number of reported violations ranged from 123 (2006) to 40 (2008). The total of 220 violations was reported for harness horses, and 193 for thoroughbred. The most frequent violations include the following substances: phenylbutazone (111), flunixin (44), cocaine (34), TCO_2 (33), furosemide (25), ergonovine and DMSO (21 each), O-desmethylpyrilamine (13), cromolyn, diclofenac and indometacin (9 each), isoxsuprine (7), acepromazine, (6), methocarbamol and procaine (5 each), naproxen (4), ketorolac (3), etorphine, lidocaine, and morphine (2 each). One violation of each was reported for the following drugs: acetaminofen, buprenorphine, carprofen, chloropromazine, codeine, desipramine, fluoxetine, glycopyrolate, guaifenesin, hydromorphone, imipramine, meperidine, mepivacaine, methamphetamine, nalbuphine, nalorphine, oxazepam, oxymorphone, phenobarbital, phentermine, prednisolone, prednisone, promazine, tramadol, and verapamil.

In conclusion, this presentation has demonstrated that while only a very small number of violations of the total tested samples were reported, a greatly varied pattern of performance enhancing drugs and substances in Illinois horse racing was revealed.

Equine Doping, Drugs, Forensic Toxicology

K34 Concentrations of Amphetamine and Morphine in Femoral Blood in Overdose Deaths Compared With Venous Blood From DUID Suspects

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After attending this presentation, attendees will learn about two of the major drugs of abuse in Sweden (amphetamine and heroin) and gain firsthand knowledge about the concentrations of these substances in blood in overdose deaths as well as in people arrested for driving under the influence of drugs (DUID suspects). This presentation compares and contrasts the concentrations of two major recreational drugs, namely amphetamine and morphine (derived as a metabolite of heroin), in peripheral blood samples from the living and the dead.

This presentation will impact the forensic science community by enabling medical examiners and toxicologists to compare the concentrations of amphetamine and morphine in blood. The large size of the present material, the sampling and analysis of drugs in peripheral blood (femoral or venous) and use of modern analytical methods are some of the major strengths of this research. The results will impact attendees when they are called upon to interpret drug concentrations in overdose deaths or to testify in court in cases of drug-impaired driving.

Interpreting the concentration of drugs determined in postmortem blood in terms of acute toxicity and whether an overdose and a drug poisoning was a likely cause of death is fraught with difficulties. The circumstances surrounding the death, the police reports, eye-witness statements, findings at the scene, and the autopsy all need careful consideration. People differ widely in their response to the same dose of a drug depending on factors such as absorption rate, dosage form, routes of administration, ethnicity, enzyme polymorphism, least previous experience with the drugs in question, and the development of central nervous tolerance.

Unlike in the United States where methamphetamine is the preferred central stimulant amine subjected to abuse, in Sweden it is the primary amine amphetamine that has topped the list of illicit drugs over many decades. Elevated blood-amphetamine is a common finding in postmortem (PM) toxicology as well as in apprehended drivers. Information about amphetamine concentrations in the living and the dead was retrieved from a forensic toxicology database (TOXBASE) using a cut-off concentration for positive results of 0.03 mg/L. Amphetamine was determined in blood by isotope-dilution GC-MS. The use of heroin was verified by identification of the unique metabolite 6-monoacetylmorphine (6-MAM) in blood or urine. The poisoning deaths were identified from ICD-9 codes assigned by the medical examiner and then sorted according to whether these were mono-intoxications or poly drug users.

The mean (median) and upper 95th percentile concentration were 2.0 mg/L (1.5 mg/L) and 4.2 mg/L respectively for N = 36 mono-intoxications involving amphetamine. These findings compare with 1.6 mg/L (0.4 mg/L) and 4.3 mg/L, respectively for N = 383 poly-drug amphetamine-related deaths. The victims of amphetamine poisoning were mainly men (72-86%) and those in single-drug deaths were 13

years older than poly-drug deaths (48 y vs. 35 y). The median concentration of amphetamine in mono-intoxication deaths was four time higher than that of poly-drug users. The median concentration of amphetamine in blood of impaired drivers as the only drug was 0.9 mg/L compared with 0.6 mg/L in poly-drug DUID suspects. The DUID suspects had higher median B-amphetamine concentrations compared with medical examiner cases who were poly-drug users (0.4 mg/L). Regular use of amphetamine leads to tolerance although unusually high concentrations of this stimulant can be tolerated without a fatal outcome.

When forensic toxicologists report morphine in submitted blood-samples this is usually taken to mean abuse of heroin, arguably the most dangerous recreational drug. In this study the presence of 6-MAM in blood or urine was used as a biomarker for recent use of heroin. In the autopsy material, most victims of heroin-related deaths were men (88%) although there was no gender difference in their age (mean 35 y). In traffic cases, 91% of heroin users were men and they were on average two years younger than the women (33 y v 35 y). The use of heroin was identified using an analytical cut-off concentration for 6-MAM in blood of 0.005 mg/L the same as that for B-morphine. Both opiates were determined in blood and urine by isotope-dilution GC-MS.

In medical examiner cases (N = 766), the median B-morphine concentration in heroin-related deaths was 0.24 mg/L, which compares with a median of 0.15 mg/L (N = 124) in apprehended drivers with 6-MAM in blood. The concentration distributions of B-morphine in the living and the dead overlapped to a large extent. In medical examiner cases, 65% of the victims had a B-morphine concentration > 0.2 mg/L compared with 36% in the DUID cases. In traffic cases when 6-MAM was present in urine (N = 1950) but not in blood, the median B-morphine concentration was considerably lower (0.03 mg/L) and only 3.6 % had a concentration exceeding 0.2 mg/L. The presence of 6-MAM in urine but not in blood means that more time has elapsed after the last use of heroin and consequently the concentrations of morphine in blood decreases through metabolism. It was found that the concentrations of morphine in blood (median values) were remarkably similar in mono-intoxication deaths (0.25 mg/L, N = 63), poly-drug deaths (0.24 mg/L, N = 703), and in heroin overdose deaths (0.25 mg/L, N = 669); and also when the person died in some other way than drug poisoning (0.23 mg/L, N = 97).

Interpreting the concentrations of drugs in postmortem toxicology is complicated because the results do not reveal any information about the extent of prior exposure and the development of tolerance. When it comes to acute toxicity of opiates, the loss of tolerance is perhaps the most important determinant of an overdose death, especially when the drugs are administered intravenously. The results from this study show that the concentration of amphetamine and morphine in forensic blood samples cannot be used *per se* to conclude that death was the result of drug poisoning. Toxicology results should not be interpreted in a vacuum and the autopsy findings including histology, the police investigation, knowledge of the deceased person's drug habits, as well as witness statements all need to be considered when the cause and manner of death are assigned.

Amphetamine, Morphine, Blood

K35 LC/MS/MS Determinations of Hydrocodone and Hydromorphone in Oral Fluid, Urine, and Hair After Short-Term Therapy

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After attending this presentation, attendees will learn about LC/MS/MS-based opiate confirmation method in specimens from a subject who took hydrocodone over a few days for post-surgical pain.

This presentation will impact the forensic science community by providing a clearer view for interpreting forensic toxicology casework by distinguishing the profile of a therapeutic user versus one who abuses their medication.

This presentation illustrates a controlled system of how therapeutic doses of hydrocodone is distributed and detected in oral fluid, urine, and hair specimens. Attendees will learn about an LC/MS/MS-based opiate confirmation method in specimens from a subject who took hydrocodone over a few days for postsurgical pain. A comparison and contrast of these amounts will be made with those observed in DUI and DFSA casework.

Hydrocodone is a semisynthetic opiate indicated for acute and chronic pain relief. The hepatic enzyme CYP2D6 transforms it into hydromorphone and other metabolites, which follows an average serum half-life of 3.8 hours. Due to its side effects such as euphoria, sedation, and availability, hydrocodone is now one of the most commonly abused prescription drugs. It has become a common analyte in forensic toxicology confirmations, as well as controlled substance submissions from diversion and illicit use.

Most opiates are easily detected by immunoassay screens in blood, oral fluid, or urine. Modern techniques such as LC/MS/MS are very sensitive and selective in distinguishing hydrocodone from other opiates and determining the concentration. Therapeutic hydrocodone concentrations in blood typically range from 0.01-0.03 mg/L, rise to 0.10-0.20 mg/L in abusers, and plateau around 0.30-0.40 mg/L in cases of acute fatal overdose. Urine levels can be more difficult to interpret due to the inherent influences of diet, excretion patterns, and other factors.

Data on a subject who took therapeutic amounts of hydrocodone over a span of a few days will be presented. The times of doses and specimen collections were recorded and reconciled with confirmations by LC/MS/MS without an initial immunoassay screen. The extraction method for urine is a solid-phase extraction, whereas our oral fluid and hair extractions are simply diluted and filtered samples. Each type of curve provides reportable concentrations between 10 and 2,000 ng/mL. The limit of quantitation is 10 ng/mL, while the limit of detection is an administrative cutoff at 5 ng/mL.

For the therapeutic user in this case, the concentrations of hydrocodone in oral fluid remained between 0.001-0.01 mg/L, while hydromorphone levels remained at an undetectable level. In urine, hydrocodone levels were 0.01-0.50 mg/L and hydromorphone levels were within the range of 0.002-0.003 mg/L. When normalized to dosing and time, the hydrocodone and hydromorphone levels displayed a consistent ratio of concentrations between each other. In distinction, a retrospective analysis of hydrocodone in DWI and DFSA urine specimens gave hydrocodone concentrations from 0.02-73 mg/L and hydromorphone levels of 0.005-0.69 mg/L.

Hair was also examined, where the therapeutic user began submitting haircut specimens after three weeks and continued to provide clippings each week thereafter. In a sense, it is a reverse segmental analysis because the most distal ends opposite the root were sampled each week for opiate contents. Results showed an initial 25 pg/mg concentration at 18 days, which gradually peaked at 71 pg/mg after 52 days and rapidly declined in the following weeks.

These results support this methods for analyzing opiates in oral fluid, urine, and hair by LC/MS/MS. The data also provides a clearer view for interpreting forensic toxicology casework by distinguishing the profile of a therapeutic user versus one who abuses their medication.

LC/MS/MS, Hydrocodone, Hydromorphone

K36 The Uncertainty of Hair Analysis for Drugs and Poisons

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After attending this presentation, attendees will appreciate the various factors that contribute to uncertainty in interpreting results of hair analyses for drugs and poisons.

This presentation will impact the forensic science community, as well as, the field of forensic toxicology by providing a better understanding of the significance that hair growth, collection, external contamination, and the uncertainty in quantitative measurements play in interpreting the results of hair analyses for drugs and poisons.

Analysis of hair for drugs, poisons, and their metabolites has been widely reported in the scientific literature over the past two decades. There are a number of fundamental assumptions in interpreting results of these analyses including: (1) an average linear growth rate of hair of 1 cm per month; (2) sample collections occur with the hair being cut directly next to the scalp; (3) external contamination can be differentiated from ingestion; and (4) differences in measured concentrations between hair segments indicate a change in exposure.

This presentation will evaluate each of the above assumptions and provide useful information to help the attendee fully appreciate how measurement uncertainty plays an important role in interpreting the results of hair analysis for drugs and poisons. The results of a thorough review of hair growth studies will be presented and a more realistic growth rate of 1.4 ± 0.5 cm/month will be proposed. Separately, the results of a hair collection study will be discussed. The results of this study suggest that an average of 0.8 ± 0.2 cm of hair may be left on the scalp after collection, corresponding to 0.6 ± 0.3 months of hair growth. The current status of the effect that external contamination may have on positive findings in hair will be addressed. Finally, the role that measurement uncertainty of quantitative results will be addressed with examples provided that demonstrate the limitations that uncertainty presents in assessing concentration differences between hair segments.

Measurement Uncertainty, Hair, Segmental Analysis

K37 Analysis of Cocaine Analytes in Human Hair: Ultrastructural Evaluation of Human Hair by Microscopy for the Determination of Morphological Differences Following Surface Contamination and Laboratory Decontamination

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After attending this presentation, attendees will understand the morphological structure of different human hairs, the permeability of hairs and potential variations in drug absorbance, and how processes and procedures used by hair drug testing laboratories may affect hair morphology and consequently drug analyte concentrations in hair.

This presentation will impact the forensic science community by providing a better understanding of the relationship between hair morphology and the permeability of hair to drugs.

Introduction: The factors affecting the permeability of hair to drugs are not fully understood. In order to improve the analytical tools used in hair drug testing and better interpret the meaning of test results from that testing, research that examines the deposition of drugs onto hair, the factors that can contribute to drug deposition onto hair, and the role of environmental drug contamination is needed. If it is shown that hair color and or structure influences drug permeability, the current drug testing methods and interpretations may need to be modified in order to take these variations into account and remove any potential for bias and or unjustified accusation. The goal of this research is to examine the permeability of different hair types (color and ethnicities) to cocaine analytes by utilizing microscopy to help understand the relationship between hair structure and the extent of drug absorption.

Methods: Hairs (Caucasian light and dark hair types, African American; n=12 each) were contaminated with cocaine HCl powder. The structural differences between the hairs of the different types and ethnicities were visually examined before and after contamination and washing. Hairs from each sample were examined employing a variety of microscopy techniques including scanning electron microscopy (SEM), freeze fracturing combined with SEM, fluorescence microscopy, and brightfield microscopy. During fluorescence and brightfield microscopy, hairs were stained with methylene blue and rhodamine B and the extent of stain penetration examined.

Results: Multiple images were taken of each sample during examination with each microscopy technique and compiled into individual portfolios for comparison. Rhodamine B and methylene blue produced similar staining patterns when observed with bright field and fluorescent microscopy. Due to variations in excitation wavelengths, Rhodamine B fluoresced significantly better than methylene blue when examined with fluorescence microscopy. Significant differences were observed not only between hairs of different ethnicities, but between hairs within a single ethnicity as well. Deposition of dye was largely associated with the cuticular scale edges. In hair with damage or missing cuticle, the cortex was strongly stained. The thickness and number of cuticular scale layers were also examined between individuals and between ethnic groups. The SEM examination revealed ultrastructural details of the relationship between the cuticle and cortex, and demonstrated a wide variability in cuticle forms and delamination from the main hair body.

Conclusion: These preliminary data suggest that the collection of structural information from microscopic examination of hair may allow for the observed differences in hair morphology to be applied to differences in the permeability of hair to drugs. The information from this study may be useful to improve laboratory procedures employed by hair drug testing laboratories.

Hair Morphology, Drug and Dye Incorporation, Microscopy

K38 Analysis of Cocaine Analytes in Human Hair II: Evaluation of Different Hair Color and Ethnicity Types Following Surface Contamination and Laboratory Decontamination

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After attending this presentation, attendees will understand: (1) the in vitro model of drug surface contamination used to investigate cocaine analyte concentrations and ratios in hair; (2) the permeability of hairs and potential variations in drug absorbance in different hair color and types; and (3) how processes and procedures used by hair drug testing laboratories may affect drug analyte concentrations in hair.

This presentation will impact the forensic science community by directly affecting policy implementation for forensic applications of hair testing, such as the investigation of drug facilitated crimes and workplace drug testing.

Introduction: The mechanism(s) of permeability of hair to drugs are not fully understood. Research data suggest that hair color may affect cocaine's incorporation into and retention in the hair matrix. The possibility that because of hair color one individual may be more likely to test positive for a drug than another, despite both having ingested or having been exposed to the same amount of a drug, greatly concerns policymakers and forensic practitioners. The potential for such bias must be understood to ensure the correct interpretation of results and the appropriate use of hair testing. If it is shown that hair color influences drug permeability, current drug testing methods may need to be improved in order to take these variations into account and remove any potential for bias and false-positive results. The goal of this study was to evaluate cocaine analytes in hair of different color (e.g., light, dark) and ethnic origin (e.g., Caucasian, African American) after the hair has been subjected to surface contamination with cocaine and subsequent laboratory decontamination.

Methods: The *in vitro* surface contamination study design was modified to a shorter collection time, but generally followed a previously published method by Stout et al. 2006. Briefly, verified drug-free head hair samples (Caucasian light and dark hair types, African American; n=12 each) were collected under IRB protocol, contaminated with cocaine HCl powder, shampooed daily for 8 weeks with aliquots removed weekly for decontamination (two washing protocols: methanol and extensive phosphate buffer) and cocaine analyte testing by LC/MS/MS. Quantitative analytical procedures for the determination of COC, BE, CE, and NCOC in hair were performed on an Agilent Technologies 1200 Series liquid chromatography system coupled to a 6410 triple quadrupole mass spectrometer, operated in positive ESI mode. For confirmation, two transitions were monitored and one ion ratio was determined which was acceptable if within 20% of the ratio of known calibration standards. The limits of quantitation (LOQ) for COC was 25 pg/mg and BE, CE, and NCOC were 2.5 pg/mg. The upper limit of linearity was 55,000 pg/mg for cocaine and 1,000 pg/mg for all other analytes. Between run imprecision for COC at 150 pg/mg was less than 3% and at 15 pg/mg for all other analytes was less than 8%.

Results: While previous cocaine surface contamination studies were designed to provide an estimate of interindividual variation, this study included sufficient samples to determine differences between ethnic groups or hair color with statistical significance. The preliminary data suggests there was no apparent simple relationship between concentration and hair color by this *in vitro* cocaine surface contamination model.

Conclusion: The results of this study along with continued studies may influence how hair testing results are interpreted, and could have a significant impact on whether national agencies use hair testing.

Hair, Cocaine Analytes, LC/MS/MS

K39 Investigation of the Effect of Vinegar on Oral Fluid Drug Testing: Effects on Immunoassay Screening

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After attending this presentation, attendees will learn about: (1) the effects of different types of vinegars on the Orasure Intercept®, and microplate screen; and (2) the new Concateno Certus™ oral fluid collection devices with homogenous immunoassay screen.

This presentation will impact the forensic science community deomonstrating how the Orasure Intercept® oral fluid collection device exhibited many oral fluid false positive after the consumption of various types of vinegar.

Introduction: Oral fluid (OF) drug testing has become increasingly popular during recent years as an alternative matrix for drugs of abuse (DOA) testing. OF is simple and easy to collect and offers a non-invasive means of sample collection that can be applied for use in the work place, hospitals, drug treatment centers, and roadside. Although numerous studies have been published in relation to OF drug detection and identification, little work has been undertaken to investigate the effects of substances. In a separate study, several different foods and beverages and the result from this indicative study inferred the possibility that vinegar could cause an effect on an immunoassay screen were evaluated. This study was conducted to look at this effect in greater detail. This study investigates the effects of different types of vinegars on the Orasure Intercept® and microplate screen and the new Concateno Certus™ OF collection devices with homogenous immunoassay screen.

Method: Non-drug using human volunteers were asked to swirl 5mL of selected vinegars around the mouth. These included malt, white distilled, balsamic, red wine, and white wine vinegar. After consumption, OF was collected using the Orasure Intercept® or the new Concateno Certus™ OF collection devices a) immediately after mouth emptying and b) 10, 20 and 30 minutes after mouth emptying. Each volunteer provided samples using both devices for all vinegars tested. The volume, pH and time for collection of samples were recorded. OF samples were subsequently analyzed using two different immunoassays for Amphetamine, Methamphetamine, Cocaine, Methadone and Opiates. Intercept® samples were analyzed using Orasure microplates and Certus™ samples were analyzed using the Concateno homogeneous assays to observe whether the substances affected the immunoassay screening systems.

Results: The Intercept® device collected an average of 0.55 mL in the 3 minutes recommended by the manufacturer. The Certus™ device has a built-in indicator and we collected an average of 1.15 mL in an average time of 1.67 minutes. The OF pH was not affected for either collection device. Presumptive positive Intercept®/Orasure samples were observed for amphetamine, methamphetamine and cocaine following consumption of all types of vinegar. Most presumptive positives were seen at the early time points although a significant number we also observed out to 30 minutes. The screen positives were submitted for GC/MS confirmation and found to be confirmation negative – screen false positives. There was also a depression of the binding for Intercept®/Orasure samples for opiate and methadone assays although this was not enough to trigger a positive response against the kit cut off. By comparison the Certus™ /Concateno samples were negative for all vinegar types and time points.

Conclusion: The Orasure Intercept® OF collection device exhibited many OF false positive after the consumption of various types of vinegar. There was no significant difference between collection times, pH, or volume collected. False positive samples out to 30 minutes were a surprising observation. The Concateno Certus™ OF collection device was shown to collect larger volumes of fluid, more consistently, in a shorter time frame. All of the Certus™ screens were negative.

Oral fluid drug testing is increasing in popularity in forensic, clinical, and workplace scenarios. In order to avoid potential miscarriages of justice or misinterpretation of results, it is essential that any tests employed for human oral fluid drug screening should provide results that are accurate. Personnel using oral fluid drug testing devices should be aware of possible interactions that could provide false positive results. This presentation highlights the potential for false positive screening results that may be observed following the collection of oral fluid after the use of certain food types.

Oral Fluid, Concateno, Orasure

K40 Drugs and Driving Special Scientific Session: Current Research Related to Drug-Impaired Driving

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After attending this presentation, attendees will have a greater understanding of the prevalence of drug use in drivers and the latest research findings in the area of drug-impaired driving.

This presentation will impact the forensic science community by enhancing the understanding of the extent of the drug-impaired driving problem and providing specific research findings related to several drugs' effects on the skills required to safely operate a motor vehicle.

Introduction: A large proportion of the population habitually drives while taking medical and/or recreational drugs. This special session will provide information on several research aspects of drug impairment including surveys that reveal the prevalence of drugs in United States and European drivers, how drugs may be categorized by the impairment they cause, recent research findings for specific drugs using psychopharmacological tests and a drugged-driving case study involving Tizanidine with assessment by a drug recognition officer.

Topics Include: *"National Roadside Survey 2007: Results from Paired Specimens of Oral Fluid and Whole Blood," Christine Moore, PhD*. This presentation provides an overview of the results pertinent to drugs detected in paired oral fluid – blood specimens from the National Roadside Survey (2007) that was conducted at selected sites across the United States. From night-time drivers, 5,869 oral fluid samples (OF) and 3,276 blood samples were taken. Of the paired specimens, 559 pairs showed at least one matrix as drug positive; 326 pairs were positive in both matrices.

"DRUID Project: Epidemiology Studies of Drug Prevalence in Europe," Alain Verstraete, PhD. Recent epidemiology data from surveys carried out in the European DRUID (Driving under the Influence of Drugs, Alcohol and Medicines) project to assess the prevalence of alcohol and other psychoactive substances in drivers in general traffic (13 countries) and drivers involved in injury accidents (6 countries) will be presented.

"DRUID Project: A Classification System for Impairing Drugs," Alain Verstraete, PhD.
The classification and labeling of medicinal drugs according to their influence on driving performance is one of the work goals for DRUID. Dr. Verstraete will present the latest progress on this difficult task.

"Psychomotor and Mood-Altering Effects of CNS-Active Adjuvant Drugs Used in Treatment of Chronic Nonmalignant Pain, Alone and in Combination with Oxycodone in Healthy Volunteers," James P. Zacny, PhD. Very few research studies have studied the effects of carisoprodol or pregabalin on psychomotor abilities. Research on the combination of different drugs is even harder to find. A battery of psychopharmacological tests will be detailed along with the results of those tests on healthy volunteers dosed with carisoprodol and pregabalin, with and without an oral opioid on board.

"Psychomotor and Mood-Altering Effects of Oxycodone and Ethanol, Alone and in Combination, in Healthy Volunteers," James P. Zacny, PhD. Opioid use in the general population has escalated dramatically in recent years, and the incidence of opioids detected in drivers has followed a similar pattern. Dr. Zacny will present the results of an interaction study involving oxycodone and alcohol in healthy volunteers.

"Analytical and Interpretation Challenges in a Tizanidine DUID Case," Amy Miles, BS. Tizanidine is a short-acting muscle relaxer used for the treatment of muscle spasticity. In this case history the subject was stopped for impaired driving. A Drug Recognition Expert (DRE) was called in approximately an hour later to perform the 12-step evaluation. Upon completion of the evaluation the DRE was unable to find any impairment but concluded the subject was impaired at the time of the stop. The case will be discussed in detail including a review of the analytical challenges posed by this short-acting drug.

Drugs and Driving, Impairment, Forensic Toxicology

K41 Direct LC/MS/MS Quantification of Plasma Cannabinoids and Their Glucuronides

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After attending this presentation, attendees will be able to describe an LC/MS/MS method for the simultaneous identification and quantification of THC, its Phase I metabolites, 11-hydroxy-THC (11-OH-THC) and 11-nor-9-carboxy-THC (THCCOOH), other cannabis constituents, cannabidiol (CBD) and cannabinol (CBN), and its Phase II metabolites, THC-gluc and THCCOOH-gluc in 0.5 mL human plasma.

This presentation will impact the forensic science community by offering a novel analytical method for sensitive and specific

simultaneous quantification of both Phase I and II cannabinoid metabolites in a single plasma extract.

Introduction: Cannabis is the illicit substance most commonly detected in blood of driving under the influence of drugs (DUID) cases and in fatally injured drivers. Cannabinoid glucuronides have been proposed as potential markers of recent cannabis intake; however, to our knowledge, no method that directly detects and quantifies Δ^9-tetrahydrocannabinol (THC) and its metabolites THC-1-glucuronide (THC-gluc) and 11-nor-9-carboxy-THC glucuronide (THCCOOH-gluc) in plasma has been reported.

Method: Cannabinoids were extracted from 0.5 mL human plasma following pH adjustment with 1.5 mL 2% ammonium hydroxide (v/v), with reversed-phase polymeric SPE cartridges. Samples were reconstituted in 150 uL mobile phase consisting of 70% A (10 mM ammonium acetate, pH 6.15) and 30% B (acetonitrile). 30 uL was injected onto a LCMSMS instrument consisting of a Shimadzu SIL-20ACXR auto-sampler, DGU-20A3 de-gasser, LC-20ADXR pumps, and CTO-20AC column oven interfaced with an Applied Biosystems 3200 Qtrap mass spectrometer equipped with a TurboV ion source operated in electrospray mode. Gradient chromatographic separation was achieved utilizing a Restek Ultra II Biphenyl HPLC column (100 x 2.1 mm, 3 µm particle size) with a 0.4 mL/min flow rate and an overall run time of 9 min. Detection and quantification were conducted in MRM mode with THC-gluc, THCCOOH-gluc, 11-OH-THC, THCCOOH and CBD ionized in negative polarity mode while CBN and THC were ionized in positive polarity mode.

Results: Limits of quantification (LOQ) were 0.5 ng/mL for THC-gluc, 1.0 ng/mL for THC, CBD, CBN, 11-OH-THC and 5.0 ng/mL for THCCOOH and THCCOOH-gluc. Calibration curves were 0.5-50 ng/mL for THC-gluc, 1-100 ng/mL for THC, CBD, CBN, 11-OH-THC and 5.0-250 ng/mL for THCCOOH and THCCOOH-gluc ($r^2 > 0.990$ and concentrations ±15% of target, except at the LOQ where ±20% was acceptable). Validation parameters were tested at three concentrations spanning the linear dynamic range. Inter-day recovery (bias) and imprecision (N=18) were 100.0-108.0% of target concentration and 2.3-8.0% relative standard deviation (RSD), respectively. Extraction efficiencies were 67.6 – 91.8%. Matrix effects ranged from -56.2 – 89.9%, depending on the analyte, with negative values indicating ion suppression; matrix effects at each quality control concentration were similar for native and corresponding deuterated compounds enabling low QC quantification within 88.1-117% of target concentration (N=18). Similar matrix effects were observed for twelve different blank plasma sources fortified with low quality control concentrations. Analyte stability was assessed under the following conditions: 24 h at room temperature, 72 h at 4°C, three -20°C freeze-thaw cycles, and 24 h on the 4°C autosampler; losses of less than 17.9% were observed for each condition, except for THCCOOH-gluc that experienced losses up to 25.2% during storage for 24 h at room temperature. No quantifiable analyte carryover was observed at two times the upper LOQ.

Conclusions: A chromatographic method for the identification and quantification of cannabinoid metabolites in human plasma is described. This method will be employed in ongoing cannabinoid administration studies and will be useful for in assessing plasma cannabinoid concentrations in clinical toxicology and DUID cases.

Supported by the Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health.

Cannabinoids, Glucuronides, LC/MS/MS

K42 Cannabinoid Concentrations in Daily Cannabis Smokers' Oral Fluid During Prolonged Monitored Abstinence

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After attending this presentation, attendees will: (1) be able to characterize THC and metabolite oral fluid disposition in daily cannabis smokers during monitored abstinence; and, (2) will be able to understand cannabinoid oral fluid detection windows for interpretation of oral fluid cannabinoid results.

This presentation will impact the forensic science community by improving the interpretation of oral fluid test results from chronic, daily cannabis smokers.

Introduction: Oral fluid is an increasingly popular alternative matrix for drug treatment, workplace, and driving under the influence of drugs testing programs due to ease of collection and reduced opportunity for specimen adulteration. The National Highway and Safety Administration's 2007 National Roadside Survey reported that more than 14% of nighttime drivers' oral fluid tested positive for potentially impairing drugs; Δ^9-tetrahydrocannabinol (THC), the primary psychoactive component of cannabis, was the most commonly detected drug at 6.1%. Drug presence does not necessarily imply impairment, as THC and metabolites were detected in chronic cannabis smokers' plasma for up to seven days during monitored abstinence.

Methods: Healthy male daily cannabis smokers provided written informed consent to participate in this IRB-approved study. Participants resided on a closed research unit with continuous monitoring for up to 31 days. Oral fluid specimens were collected once each 24 h with the Quantisal™ collection device, and analyzed for THC, cannabidiol (CBD), cannabinol (CBN), and 11-nor-9-carboxy-THC (THCCOOH) by two-dimensional (2D) GCMS. Limits of quantification (LOQ) were 0.5 ng/mL for THC and CBD, 1 ng/mL for CBN (electron impact 2D-GCMS), and 7.5 pg/mL for THCCOOH (negative chemical ionization 2D-GCMS).

Results: Seventeen cannabis smokers (19-43 years old) reported current smoking of 1-18 joints/day (median 9 joints/day), and up to 45 joints/day during peak use. Participants resided on the closed residential unit for 5 to 31 days. Cannabinoids were quantified in 304 oral fluid specimens. Maximum THC, CBD, and CBN concentrations occurred upon admission, while THCCOOH concentrations generally peaked within the first two days of abstinence. All specimens from one subject who spent 30 days on the research unit were below LOQ; however, self-report data indicated only 1 joint/day typical use. THC was quantifiable in only 26 specimens (8.6%) at concentrations <82.5 ng/mL, and was never present without concurrent THCCOOH. Daily THC detection rates in Quantisal™ collected oral fluid decreased from 94 to 41 to 18% during the first, second and third days of abstinence, with no THC detectable after this time. Of the specimens 6.6% were THC-positive at the recommended DRUID confirmation cutoff of 1 ng/mL and 5.3% at the proposed Substance Abuse and Mental Health Services Administration 2 ng/mL cutoff. THCCOOH detection in oral fluid was prolonged, with concentrations up to 202.5 pg/mL in 141 specimens (46.4%). All 17 participants resided on the secure research unit for at least five days, with 81.6% of daily THCCOOH concentrations ≥LOQ. During the first, second and third weeks of abstinence, 74.6, 41.3, and 29.6% of specimens were THCCOOH positive. For the 61 specimens

collected on days 22-31, 14.8% remained ≥7.5 pg/mL. CBD (initial concentrations 2.6 - 3.4 ng/mL) and CBN (1.1 - 5.2 ng/mL) were only detectable on the first day in oral fluid from two and eleven subjects, respectively.

Conclusions: THC and THCCOOH were detected in oral fluid of chronic, daily cannabis smokers during monitored abstinence for 3 and 30 days, respectively. The differences in detection windows may be attributed to the much lower LOQ for THCCOOH. For the first time, it is documented that detection of cannabinoids in oral fluid of daily cannabis smokers may not reflect recent use and may not indicate impairment in daily chronic cannabis smokers. Neurocognitive impairment in daily chronic cannabis smokers was demonstrated on some measures for 7 to 28 days. Additional research is needed to determine if cognitive and performance impairment persists during the detection window of cannabinoids in oral fluid. It is hypothesized that residual THC in the brain produces the performance impairment observed in chronic daily cannabis smokers. These novel cannabinoid oral fluid data are important for interpreting oral fluid test results.

This research was funded by the Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health.

Oral Fluid, Cannabinoids, Chronic Cannabis Smoking

K43 Effects of Smoking the Synthetic Cannabinoids JWH-018 and JWH-073 on Human Performance and Behavior: Controlled Administration and DUID Case Reports

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After attending this presentation, attendees will be able to identify the drugs present in herbal "legal high" smoking blends, and describe the effects on subjects' behavior and performance following smoked administration.

This presentation will impact the forensic science community by describing the behavior of subjects under the influence of synthetic cannabinoids drugs, their performance in field sobriety tests, and observations of actual subjects arrested for DUI after using these products.

Herbal materials sold as "legal highs" containing synthetic cannabinoids agonists with purported effects similar to tetrahydrocannabinol (THC) have been appearing on the United States recreational drug market since 2008. The most popular of these products is the K2 brand, sold as a variety of blends including Blonde, Summit, Strawberry, and a variety of others. These products have been shown to contain the cannabinoid CB_1 agonists JWH-018, and JWH-073.

A variety of K2 blends were administered to human subjects in a controlled environment, and their clinical signs, symptoms, and performance in the Drug Recognition Expert (DRE) assessment matrix were evaluated. Additionally, driving performance and actual behavior of subjects arrested under suspicion of DUID after smoking K2 are reported and discussed.

Ethical review was provided by the University of Central Missouri Institutional Review Board. They approved an experiment involving the administration of low doses via smoked route of administration of K2 blends containing JWH-018 and JWH-073 to six subjects. Subjects were screened and determined to be drug free prior to administration. Subjects were administered either one or two inhalations of K2 from a water pipe. Their response and vital signs were monitored by medical personnel. Subjects completed the DRE evaluation before and after administration of the drug. Blood, urine, and oral fluid were collected from subjects for later analysis. Analysis was performed by liquid chromatography tandem mass spectrometry (LCTMS), with a limit of detection of 0.1ng/mL in blood for the parent compounds.

Subjects began to feel the effects of the drug within 2-3 minutes of administration. Effects included increased pulse and blood pressure, dry mouth, bloodshot, watery eyes, and lack of convergence. Subjects reported a variety of subjective effects, including lightheadedness, blurred vision, motor restlessness, some mild agitation, and temporal disorientation. Four of six subjects indicated some mild anxiety. Subjects indicated a preference to continue dosing, although this was not permitted. Those subjects who were experienced marijuana users described the experience as qualitatively inferior to marijuana. Subjects' performance in field sobriety and psychophysical tests was variable. Some subjects demonstrated loss of balance, impaired coordination, and difficulty following instructions. Others displayed negligible performance effects following this low dose. The acute effects diminished between two and four hours following administration. Three of six subjects indicated high levels of fatigue once the acute effects had worn off.

Blood and oral fluid collected within an hour of administration were positive for both JWH-018 and JWH-073. Urine was positive for glucuronidated monohydroxy-, dihydroxy-, and trihydroxy- metabolites of both parent compounds. In one subject urine continued to test positive for metabolites for up to 24 hours post-administration.

Data was reviewed for several subjects arrested for suspected DUI following use of K2. Subjects displayed similar symptoms including increased pulse and blood pressure, bloodshot eyes, lack of convergence, and poor performance in field sobriety tests.

The synthetic cannabinoids contained in K2 cause marijuana-like effects on subjects' psychophysical response and driving performance.

Synthetic Cannabinoids, K2, Driving

K44 Corresponding Impairment With Hydrocodone and Driving Under the Influence Investigations

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After attending this presentation, attendees learn more information about the impairing effects of hydrocodone, alone, and in combination with illicit and legal drugs for the purposes of driving.

This presentation will impact the forensic science community by providing a possible tool for court when testifying in DUID cases.

There has extensive debate over whether opiates, especially Hydrocodone, impair driving. Publications have not yet examined driving behaviors, driving under the influence (DUI) cases, and drug recognition evaluations (DRE) where toxicological results indicated hydrocodone was present. A retrospective three year study of DUI and DRE investigations involving hydrocodone, in Orange County, CA, was conducted. Driving behavior, the number of cues missed on the validated Standard Field Sobriety Tests (SFST), the demeanor of the driver, speech pattern, eye response, and physical appearance were evaluated. From over 150 cases evaluated, five cases were observed with only hydrocodone present. However, there was a high correlation between the officer reported impairment and hydrocodone when combined with muscle relaxants (n=81) and/or benzodiazepines (n=103). The most frequently occurring muscle relaxant was carisoprodol with alprazolam and diazepam as the most prevalent benzodiazepines. It was also determined that poly-drug use concurrent with hydrocodone was not limited to prescription drugs. An increased rate of vehicular collisions, of poor performance on psychological tests

and necessitated additional DRE evaluation was noted when hydrocodone and a central nervous system depressant were present. Furthermore, the psychological tests administered by the investigating officer appear to be a reliable measure of impairment for poly-drug cases involving hydrocodone with and without other drugs present.

Hydrocodone, DUID, DRE

K45 Fatal Intoxication Due to Trihexyphenidyl - A Case Report

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After attending this presentation, attendees will learn about trihexyphenidyl pharmacological effects, metabolism and poisoning, method of analytical detection, and autopsy findings.

This presentation will impact the forensic science community by noting that fatal poisoning with trihexyphenidyl is very rare, based on the literature data, especially when no other central nervous system depressants and/or significant pathological changes are taken into account.

Trihexyphenidyl (THP) is an anticholinergic agent with forensic toxicological interest due its frequent abuse and reported overdose, while fatal poisoning is rare. It is a potent anticholinergic drug used in the treatment of Parkinsonism and in the control of drug-induced extrapyramidal side effects. Its mode of action is preventing the effects of acetylcholine by blocking its binding to muscarinic cholinergic receptors at neuroeffector sites on smooth muscle, cardiac muscle, and gland cells, in peripheral ganglia, and in the central nervous system. Side-effects of THP include disturbance of recent memory, tachycardia, bradycardia, and can precipitate glaucoma in predisposed patients. THP-hydrochloride is well absorbed from the gastrointestinal tract producing average peak plasma levels at 1.3 h after single oral dose of 2 and 15 mg and reaching Cmax of 0.01 and 0.05 mg/l, respectively. The half-life time varies from 3.6 h up to 33 h, following multi-compartmental kinetics. THP undergoes extensive metabolism and hydroxy-THP was reported as the major metabolite present in plasma and urine. Ethanol and other central nervous system (CNS) depressants, such as anxiolytics, sedatives, and hypnotics, can increase the sedative effects of THP.

This report presents a case of a 59-year-old female with a history of paranoid disorder being treated in an outpatient program and who was found dead in the house where she lived alone. External examination of the body yielded no evidence of external injuries or violence. Autopsy findings revealed no marked pathological changes. Femoral venous blood, urine, bile, and gastric content were collected for toxicological analyses. Toxicological analysis based on gas chromatography-mass spectrometry (GC-MS) analysis revealed THP and its major metabolite (hydroxy – THP) in blood and urine. Ethanol was analyzed in femoral venous blood and urine by head-space gas-chromatography with a flame ionization detector (GC/FID).

Qualitative GC/MS analysis confirmed the presence of THP in blood and urine, hydroxy-THP in blood, urine and bile. The presence of these substances and other xenobiotics wasn't confirmed in gastric content. GC/MS quantitative analysis revealed THP concentration of 0.053 mg/L in femoral venous blood and 0.560 mg/L in urine. The blood and urine ethanol concentrations were 0.096 g/L and 0.100 g/L, respectively.

Based on these results and literature data the cause of death was determined to be THP poisoning. It is suggested that rare case of death associated with THP overdosage should be taken in conjunction with central nervous system depressants (benzodiazepines, ethanol) and/or with other pathological disorders. Thus, this case could not be supportive for this allegation. The circumstances of the case exclude homicide; however, these data are not sufficient to determine neither suicide nor accident as a manner of death.

Trihexyphenidyl, Fatal, Poisoning

K46 Postmortem Toxicological Investigation of Alcoholic Ketoacidosis

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After attending this presentation, attendees will understand the importance of performing toxicological investigations for alcoholic ketoacidosis to provide a possible cause of death in postmortem cases with no anatomical and toxicological cause of death where victim has a history of alcohol abuse.

This presentation will impact the forensic science community by providing recommendations how to establish postmortem toxicological investigations of alcoholic ketoacidosis.

Ketoacidosis is a biochemical disturbance in the body. If no glucose is available, the body will utilize fatty acids as an alternative fuel pathway and ketone bodies will be produced. The increase of ketone bodies (acetoacetate, acetone and betahydroxybutyrate (BHB) or 3-hydroxybutyrate) in the blood will lower the blood pH. Two particular forms of ketoacidosis exist, alcoholic ketoacidosis as a result of chronic alcohol abuse and diabetic ketoacidosis as a result of a reduction in insulin. In contrast to diabetic ketoacidosis in which hyperglycemia occurs, alcohol ketoacidosis produces usually a hypoglycemia although a slight hyperglycemia can exist. The symptoms for the two forms are very similar and include nausea, vomiting, abdominal pains, loss of appetite, lethargy, weakness, and unconsciousness.

In this presentation, the toxicological results of postmortem cases at the Netherlands Forensic Institute from January 2006 with no anatomical cause of death and the victim having a history of alcohol abuse were examined. The goal was to evaluate the importance of toxicological investigations for alcoholic ketoacidosis to provide a possible cause of death in such cases. Included were those cases with no anatomical cause of death, the victim having a history of alcohol abuse, and toxicological analysis of BHB. All cases were toxicologically screened for the presence of alcohol, drugs of abuse and prescription drugs.

In total six cases were included; four male and two female with age ranging between 39 and 59 years. In five cases, the bodies were found dead in their homes and in one case the victim was found by her husband at her home needing resuscitation and subsequently died in the hospital. At autopsy, the pathologist found no anatomical cause of death or clear cause of death. Toxicological analysis for the presence of alcohol, drugs of abuse, and prescription drugs resulted in no indications for a toxicological cause of death. Alcohol was detected in blood in three cases in concentrations of 0.003, 0.032, and 0.18 g/dL, respectively, and in urine in four cases in concentrations varying from 0.006 to 0.24 g/dL. In four cases, prescription drugs were found. In all cases, acetone was detected in blood, urine, or both in the standard alcohol analysis method.

Further analysis on BHB and acetoacetate in blood, urine or vitreous humor was performed to determine possible ketoacidosis and

concentrations of glucose and lactate in blood, urine, or vitreous humor were analyzed to determine possible hypo- or hyperglycemia. Based on the combined glucose and lactate levels in vitreous humor, in one case an indication for hypoglycemia was found (measured concentration lower than 7.5 mmol/L) and in another case hyperglycemia was concluded (measured concentration was 30 mmol/L). Acetoacetate could not be detected except in a low concentration in one case, because it spontaneously decarboxylates to acetone. Measured concentrations of BHB varied from 1 to 14 mmol/L in blood and from 1 to 11 mmol/L in vitreous humor. In literature, BHB concentrations are considered normal below 0.5 mmol/L, elevated up to 2.5 mmol/L, and high and pathologically significant over 2.5 mmol/L. The measured BHB concentrations in this study are all elevated or high. Because no anatomical cause of death and toxicological cause of death due to alcohol or drugs were found, it was concluded that alcoholic ketoacidosis could have contributed to death in five cases and ketoacidosis due to hyperglycemia in one case.

In conclusion, in cases with no anatomical and toxicological cause of death, a history of alcohol abuse, and the presence of acetone in blood or urine, analysis of BHB in blood and vitreous humor may provide a possible cause of death by alcoholic ketoacidosis.

Alcoholic Ketoacidosis, Beta-Hydroxybutyrate, Postmortem

K47 A Decade of Deaths in the OC Where Drugs Were Detected

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After attending this presentation, attendees will understand the compilation of information assembled from the last 10-years of postmortem cases where drugs were present. The attendees will be introduced to the use of the compilation involving cases where drugs were both associated and not associated with the coroner cause of death.

This presentation will impact the forensic science community by introducing a supplemental set of data to the currently used texts and references. The data summarizes the recent decade (2000-2009) in Orange County, California, where drugs were present in postmortem cases. The compilation of drug levels and associated types of death will provide an additional resource for toxicologists during examination and comparison against related casework.

Many current references used by toxicologists have limited numbers of subjects in order to interpret drug levels associated with postmortem cases. These references include levels from clinical and hospital submissions involving relatively few subjects or cases. To supplement this data, a compilation of postmortem investigations where drugs were detected during the 2000 to 2009 period was initiated. The results will be presented from cases involving trauma, natural death, overdose of the drug of interest, and poly-drug deaths for 52 of the most common drugs in Orange County, California (population 3 million). Furthermore, information is included from central blood, peripheral blood, and where possible, liver and brain tissue. In some of the natural and trauma cases where the drug was not the cause of death, liver or brain tissue levels are included. This additional information can assist in cases where blood is not available. Not included as a specific drug are alcohol and carbon monoxide, but these were included in the classification of poly-drug cases. The database will include half-life, structure, and other reference material for convenience, when available from literature sources.

Toxicology, Drug Level, Database

K48 Helium Detection in Postmortem Specimens

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After attending this presentation, attendees will gain knowledge about the detection of helium in postmortem specimens using headspace gas chromatography/thermal conductivity detection (GC/TCD) in cases where death by asphyxiation using helium is suspected.

This presentation will impact the forensic science community by providing a method for the detection of helium in a variety of different postmortem specimens, allowing for a toxicological confirmation for the cause of death.

Currently, in the majority of cases, helium toxicity is only listed as the cause of death based on scene investigation; common scene observations in cases of suicide by helium inhalation includes a plastic bag covering the head with a hose running from it to a helium tank.

Helium is the second lightest element and the second most abundant element in the universe after hydrogen. Its major use is in cryogenics to cool superconducting magnets, such as those used in MRI devices. It is also used as a lifting gas in balloons and airships, as well as in combination with oxygen and nitrogen for deep sea diving in order to reduce the effects of narcosis, an alteration in consciousness that can occur; the proportions of oxygen, nitrogen, and helium are adjusted depending on the circumstances. Because of its low density, it can be inhaled unconsciously, making it a potential asphyxiant under certain conditions. There have only been a few cases reported of accidental asphyxiation by helium, but over recent years, its use as a means of suicide has increased. The increase in the occurrence of suicidal asphyxiation by helium is believed to be, in part, due to certain groups and internet sites advocating helium as the preferred method of suicide because it is widely available and a quick, painless death. One of the most influential publications is "*Final Exit – The Practicalities of Self-Deliverance and Assisted Suicide for the Dying*," in several reported cases, this literature was found on the scene.

The typical signs of asphyxia include cerebral and pulmonary edema, congestion of internal organs, petechial hemorrhages, and frothy edema in the respiratory tract. These signs are sometimes present, although there are often no significant postmortem abnormalities. In cases where such a cause of death is suspected, a reliable detection method is needed.

Postmortem specimens of five cases in which helium asphyxiation was cited as the cause of death were analyzed for the presence of helium. At the time of autopsy, samples of lung, brain, and blood were collected and sealed in 22 mL headspace vials by the forensic pathologist. Three of the five blood samples analyzed were femoral, and the source of the other two samples is not known. Each specimen was analyzed using headspace GC/TCD, with separation performed at 50°C (isothermal) on an HP-Molesieve column using nitrogen as the carrier gas. The vials were incubated at 38°C for 2 minutes, and then 100 µL of headspace was removed from the vial and injected into the GC.

Helium was detected in four of the five cases analyzed. In each of the positive cases, helium was detected in the lung. Two of the cases also tested positive for helium in the brain. The limit of detection for this technique was determined using the formula LOD = Xm + 3SD, where Xm is the mean value of the peak areas for blank samples and SD is the

standard deviation of the mean value. Using this approach, the limit of detection was calculated to be a peak area of 3.13×10^5. Samples of lung and brain from cases in which the cause of death was not related to helium or any other inhalants were also analyzed and found to be negative. Confirmatory analyses are being conducted using gas chromatography/mass spectrometry (GC/MS) in order to verify GC/TCD identification of helium.

In conclusion, this analysis provides a method for detection of helium that is easily conducted, both in the acquiring of the specimen and the toxicological analysis.

Helium, Gas Chromatography, Postmortem

K49 *In Vitro* Adsorption of Carbon Monoxide and Hydrogen Cyanide in Pooled Blood

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After attending this presentation, attendees will be able to apply the findings of this study to the interpretation of results of blood carboxyhemoglobin (COHb) and cyanide (CN^-) analyses.

This presentation will impact the forensic science community by informing those who investigate accidents associated with fires of the effect that an atmosphere containing primary combustion gases—that is, carbon monoxide (CO) and hydrogen cyanide (HCN)—will have on postmortem blood from open wounds of victims.

The Federal Aviation Administration's Civil Aerospace Medical Institute (CAMI) assists in the investigation of fatal aircraft accidents by conducting toxicological analyses of specimens received from victims of the accidents. One aspect of the analyses is the determination for the presence of primary combustion gases in blood specimens. Combined with the crash site investigation, autopsy and pathology findings, and toxicological results, the investigators could determine whether the crew members were incapacitated by engine CO leaks into the cabin area, whether they survived the crash and were overcome by inhaling CO and HCN from aircraft fires, whether and/or the victims died on impact or came to a rapid death from the intense heat of the fire without inhaling these gases.

Because of the violent impacts involved in crashes, victims quite often suffer large open wounds near sites on the body from where autopsy whole blood is collected. Many aircraft crashes result in fire, which in turn, fill the atmosphere of the victims with smoke (CO and HCN). It is important to determine whether pooled blood in those open wounds may have adsorbed CO and HCN after death and could erroneously lead investigators to determine that the presence of COHb and CN^- in whole blood was the result of breathing in primary combustion gases.

A chamber was set up in the CAMI laboratory to determine whether CO and HCN may be adsorbed in undisturbed, pooled whole blood. To determine in vitro CO adsorption, a large laboratory desiccator was used as the chamber. A light film of silicone grease was applied to the valve and the rim of the lid and chamber. A female Luer-Lok fitting was affixed to the arm of the valve by use of a small piece of Tygon tubing. To facilitate air movement in the chamber, a large cross-shaped magnetic stirring bar was placed at the bottom of the chamber, which was rotated with a magnetic stirring plate. A ceramic plate with numerous rows of holes was placed above the stirring bar. Setting on it was a shallow open dish containing 4 mL of whole human blood that had been treated with sodium heparin. A 100-cc valved Luer gas syringe was used to evacuate air from the chamber and introduce pure CO into it to achieve desired concentrations. Prior to the setup, the volume of the chamber was determined by measuring the amount of water required to displace all the air in the chamber and lid, after taking into account the volumes of the blood sample and the items used in the desiccator. The chamber volume was determined to be 9038 cc. Various concentrations and lengths of CO exposure to the pooled blood were conducted. COHb concentrations were determined spectrophotometrically.

The apparatus was modified slightly for the determination of in vitro HCN adsorption by using an additional open dish containing a 5-mL beaker having a weighed amount of sodium cyanide (NaCN). The Ideal Gas Law was used to determine the amount of NaCN required to achieve the desired concentrations of HCN in the chamber. To conduct the experiment, 4 mL of heparin-treated, whole human blood was used in the second dish. With the lid of the chamber partially opened, 1 mL of concentrated sulfuric acid was added to the beaker containing the NaCN; then the chamber lid was immediately closed. The volume of the chamber was determined to be 8981 cc, after taking into account the volumes of the blood sample, sulfuric acid, and the items used in the desiccator. Two concentrations and various lengths of HCN exposure to the pooled blood were conducted. CN^- concentrations were determined colorometrically by microdiffusion; then, positives were quantitated spectrophotometrically.

No significant amount of COHb was detected in the whole blood of the experiment after exposure to CO at 5532, 8298, 11064, 22129, and 33193 ppm for 30- and 60-minute exposure times. However, CN^- concentrations in whole blood increased with exposure to an atmosphere containing HCN at 100 and 200 ppm each at 15, 30, 45, and 60 minutes of exposure times. The CN^- concentration in blood ranged from 1.55 to 5.01 µg/mL.

Therefore, there is a potential for blood CN^- levels to increase by the adsorption of atmospheric HCN present in the smoke. This study also demonstrated that the COHb in pooled blood exposed to an atmosphere containing CO within the parameters of this experiment would not alter the integrity of postmortem blood at an aircraft crash site. This selective adsorption is consistent with the solubility of HCN and insolubility of CO in water. These findings suggest that the COHb and CN^- levels should be carefully interpreted in view of the potential for selective presence of these primary combustion gases in blood.

Carbon Monoxide, Hydrogen Cyanide, Blood

K50 Levetiracetam (Keppra®) and Suicide

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After attending this presentation attendees will be educated on the effects of the drug levetiracetam (Keppra®) and will have explored its potential risk for suicide.

This presentation will impact the forensic science community by providing a detailed description of an anticonvulsant drug with relatively unknown toxicity. Only one case of drug overdose has been presented in the literature where the individual recovered with respiratory support. The North Carolina Office of the Chief Medical Examiner has two deaths from 2010 that are noted to have suicidal drug concentrations of levetiracetam.

Levetiracetam (Keppra®) is among the new anticonvulsant drugs that are replacing drugs such as carbamazepine, phenytoin, Phenobarbital, and valproic acid. Along with drugs such as topiramate, lamotrigene, and oxcarbazepine, the new drugs have been reported to have a more tolerable side-effect profile, better efficacy and an easier therapeutic maintenance. While the side-effect profile for levetiracetam has been good overall in comparison to classical anticonvulsants, there have been recognized psychiatric effects. The FDA revised the labeling of this drug in 2007 to include warnings regarding these potential behaviors. Individuals with prior psychiatric difficulties may be most at risk for possible mood changes, agitation, and thoughts of suicide.

A 48-year-old male was found dead on arrival, barricaded in his bedroom. Over thirty empty medication bottles surrounded the body. He was discovered with tape over his mouth and a suicide note in his pocket. The decedent had a past history of seizure disorder and multiple, attempted-suicidal, drug overdoses. An alkaline liquid-liquid extraction detected therapeutic levels of citalopram and cyclobenzaprine. Benzoylecgonine was detected after SPE. An extraction for acid/neutral compounds revealed elevated levetiracetam. The aorta and vena cava levetiracetam concentrations were 190 mg/L and 232 mg/L respectively.

A 56-year-old female was found dead in bed. She was last seen alive earlier that evening by her boyfriend. The decedent had several medical problems including diabetes mellitus, chronic pain, depression, and congestive heart failure requiring oxygen therapy. She was found with a broken oxygen concentrator, a bowl filled with pills, and a suicide note. The decedent had multiple prescriptions and was found with multiple drugs in her system including an elevated level of levetiracetam in the aorta blood at 35 mg/L and metaxalone at 26 mg/L.

The two cases mentioned above have added to knowledge of suicides involving levetiracetam and associated drug concentrations. While it is a known antiepileptic drug (AED), it has been studied in open-label trials for its potential to treat neuropathic pain and anxiety disorders. Because of levetiracetam's possible adverse psychiatric effects, it may be important to keep in mind the benefit-risk ratio of each patient during treatment with this medication.

Levetiracetam, Keppra, Suicide

K51 Ethylene Glycol Fatalities: A New Look at Interpretation

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After attending this presentation, attendees will have learned from five interesting cases of fatal Ethylene Glycol (EG) poisonings, where levels were characterized in blood, clots, vitreous humor, and gastric contents.

This presentation will impact the forensic science community by offering unique aspects for understanding the complexities of acute ethylene glycol intoxication.

Analysis of several ethylene glycol (EG) fatalities that emphasize the key aspects of interpreting intoxication and death will be presented. Collateral data based on witnessed accounts, a suicide journal, vitreous electrolytes and chemistries, as well as pathological calcium oxalate crystal formation is discussed and compared with published accounts.

EG toxicity is expressed in three clinical phases during a suicidal poisoning. The first stage is characterized by central nervous system depression, which occurs shortly after ingestion and lasts for several hours. This period involves drowsiness, disorientation, and confusion, where affected individuals may appear drunk. Convulsion, stupor, and coma may develop in the next stage, when ethylene glycol metabolites cause severe metabolic acidosis, cardiopulmonary manifestations, and multisystem organ failure. In the third stage, a well-known pathological feature is the formation of microscopically visible calcium oxalate crystals from the metabolism of EG into oxalic acid and calcium oxalate.

Case #1: A 50-year-old white male who drank antifreeze and was found alive but unresponsive. He was transported to the hospital and received treatment for ethylene glycol toxicity, acidosis, and myocardial infarction. He survived three more days at the hospital, but ultimately died. Postmortem examinations on the hospital admission blood revealed 5622 mg/L EG. There was no evidence of EG in further characterizations of the postmortem vitreous humor. This finding is supportive of 150 mg/L/hr clearance rate while EG was metabolized and eliminated during the 60-70 hours before death.

Case #2: A 21-year-old white male who was observed to be "drunk and sleepy" by his roommate. The roommate left for several hours and found him unresponsive on the floor upon returning. A note was found, where the decedent expressed his intent to commit suicide by drinking EG. Tests later confirmed 588 mg/L in femoral blood, 940 mg/L in vitreous humor, and 1612 mg/L in gastric contents. Increased vitreous creatinine and glucose levels support the renal failure that often accompanies EG toxicosis.

Case #3: A 25-year-old white male who had a history of two suicide attempts; once eight months earlier by heroin, and EG only 3 months before the latest ingestion. Toxicology analysis showed the presence of alprazolam, fluoxetine, and dextromethorphan/chlorpheniramine in therapeutic amounts. However, EG was 2701 mg/L in blood, 3597 mg/L in vitreous, and 5057 mg/L in gastric contents. Prior attempts at suicide by EG were affirmed by numerous calcium oxalate crystals found, which correlates with cumulative exposure.

Case #4: A 56-year-old white male who tried to commit suicide by doxylamine pills, but recovered on his own. He told a friend that he planned to kill himself by drinking EG. Several days later, he was found in a wooded area with a suicide note in his pocket. The toxicology analysis showed that EG had reached 7974 mg/L in femoral blood, 12446 mg/L in vitreous humor, and 23296 mg/L in his gastric contents. However, the pathological examination exposed only a slight number of calcium oxalate crystals in the kidneys.

Case #5: A 38-year-old white male who ingested a cocktail of Gatorade and EG. He had a history of depression and at least three failed suicidal attempts. Lab results revealed EG levels of 4200 mg/L in a blood clot and 5300 mg/L in vitreous humor. A "suicide journal" was present on the scene, where he recorded his experiences as the intoxication progressed over a few hours. This timeline was analyzed to correlate the behavioral and physical disturbances reported by the victim prior to his death.

These cases offer unique aspects for understanding the complexities of acute ethylene glycol intoxication. From strange investigative findings to comprehensive toxicology results, this report provides new insight into ethylene glycol poisoning at the time of ingestion to the autopsy examination and interpretation.

Ethylene Glycol, EG, Toxicity

K52 Postmortem Pediatric Toxicology

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After attending this presentation, attendees will gain an appreciation for the challenges unique to toxicological findings in postmortem pediatric cases. Attendees will learn interpretive guidelines for pediatric cases involving forensic toxicology in both a general and case-specific sense.

This presentation will impact the forensic science community by further delineating the interpretive aspects of toxicological findings in the pediatric population.

In this 12th Annual Special Session, pediatric cases involving toxicological findings are discussed. As a relative dearth exists of interpretive information involving toxicological findings in the pediatric population, this session is a forum to help elucidate and clarify such issues. The format is a short case presentation including pharmaco-toxicokinetic data and other relevant ancillary information followed by

audience participation to provide interpretive clarity around the case-specific impact of the toxicological findings.

Drs. Scott Denton and Tom Andrew will discuss similar cases involving comfort care of children and postmortem morphine findings. Dr. Andy Robinson will discuss a pediatric case involving methadone, Dr. Laureen Marinetti will describe a case involving the pesticide DEET, and Dr. Bruce Goldberger will present the results of a pediatric research study conducted in Afghanistan.

Pediatric, Toxicology, Postmortem

K1 Alcohol Elimination Rate Variability and Subject-Altered General Consumption of Alcohol

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Attendees of this presentation will learn about variability in alcohol (ethanol) elimination rate after alteration, if any, of the general consumption of alcohol claimed by the human test subjects.

This presentation will impact the forensic community by providing a better qualified expert opinion concerning alcohol elimination rate variability and subject-altered general consumption of alcohol beverages to assist in judicial processes.

A decrease in a person's general consumption of alcohol may decrease their rate of elimination of alcohol; conversely an increase in such consumption may increase their elimination rate. Environmental changes in general consumption of alcohol may contribute additional variability to other biological and analytical variability inherent in the alcohol elimination rate of a person.

Test subjects were obtained from a forensic population of motor vehicle drivers under direction of their legal counsel. Informed consent excluded persons seeking, or having received, counseling and/or medical treatment concerning alcohol, and those with limiting physical or mental health. Some subjects consumed a similar light breakfast hours prior to their arrival for testing and initiating alcohol dosing. After consumption of their alcohol beverages on two separate days, suitable breath samples from subjects in the elimination phase were analyzed about every 20 minutes for their alcohol (ethanol) concentration using an Intoxilyzer instrument. Volume and duration of subject breath samples were concomitantly monitored by spirometry. Instrument calibration was confirmed using forensic alcohol standards from different manufacturers with differing concentrations and commercial simulators. The alcohol elimination rate from the second test day was multiplied by the inverse of relative instrument response to that of the first day for further data analysis. Dialogue with test subjects occurred on more than one occasion, with an embedded impromptu query involving change, if any, to their general consumption of alcohol beverages.

Test subjects (65 males and 11 females) had a median age (with range) of 38 years (19–70) and were tested twice with a median of 91 (28–924) days apart. The alcohol elimination rate (mg/210 dL/hr) on the first day had a median of 17.9 (11.6–24.4). For further data analysis, subjects were divided by response to their indicated altered general consumption of alcohol into: group "zero" (51 persons that claimed no change), group "minus" (21 persons claimed decreased consumption), and group "plus" (4 persons claimed increased consumption). Some subjects in group zero were hesitant to inform of a decreased consumption from concern about an adverse inference. One subject who initially claimed a decrease indicated a contrary increase in a later discussion. The alcohol elimination rates (mg/210 dL/hr) for these groups had a respective median of: -0.07 (-3.2–2.4), -2.9 (-5.4–0.7) and 2.3 (0.4–3.2). The relative change in alcohol elimination rate had a range from -23.1% to 27.4%: the subject with the largest relative decrease had an initial high rate of 21.3 in group "minus" and occurred 56 days apart; the subject with the largest relative increase in group "plus" had the lowest initial rate of all subjects and occurred 84 days apart. Adjustments of the alcohol elimination rate for interassay variability had a median of -0.03 (-0.47–0.34). The standard deviation of alcohol elimination rate for group "zero" was 1.38 mg/210 dL/hr, with no correlation between rate variability and delay to second test.

Variability of alcohol elimination rate (mg/210 dL/hr) for subjects divided into no change (0), decreased (-) and increased (+) general consumption of alcohol with the respective group shift of 0, -2.4 and +2.4, with a subject therein with an additional inherent variability of ±3, would describe 96.0% (73 of 76) of test subjects herein: if a decrease in consumption occurred for the two exception subjects in group "zero" and no change for the one exception subject in group "minus", contrary to those subject claims, then all subjects of this study would be described. If additional rate variability of ±2 was alternatively considered, then 86.9% of subjects are described.

Alcohol, Elimination, Variability

K2 Phencyclidine (PCP) in Fatally Injured Drivers and DUID Arrests in Harris County, Texas

Fessessework Guale, DVM, Jeffrey P. Walterscheid, PhD, Terry Danielson, PhD, Ashraf Mozayani, PhD, PharmD, and Luis A. Sanchez, MD, Harris County Medical Examiner's Office, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will gain knowledge of the incidence of phencyclidine (PCP) in arrested and deceased automobile drivers in a major metropolitan area. Attendees will also be made aware as to how an appropriately broad toxicological analysis can assist in distinguishing between reckless, suicidal behavior and drug-induced intoxication.

This presentation will impact the forensic science community by educating toxicologists on the evaluation of drivers on PCP. Simple analyses for alcohol alone might not have been sufficient to interpret these cases, and observations suggest that a wider screen for drugs of abuse can provide valuable information in understanding the driver's state of mind at the time of a collision or DRE evaluation.

Phencyclidine is a dissociative anesthetic drug that induces an altered mental state at sub-anesthetic doses, where the user may experience a range of sensations from tranquility to detachment and psychosis. Since PCP is a weak base, it is well-absorbed whether it is smoked, injected, or ingested. The onset of intoxication begins within minutes of taking PCP, and can last for several hours. Residual effects may persist for days after the last dose. Its high lipid solubility allows it to accumulate in adipose and brain tissue, with a prolonged excretion interval over several weeks.

The effects of PCP severely undermine an individual's ability to drive safely, and often results in DUID arrests or fatal motor vehicle accidents. Its chemical properties allow it to be easily abused by smoking or swallowing before or during operation of a motor vehicle. An acute 5 mg dose of PCP typically causes drunken behavior with drowsiness, slurred speech, poor coordination, and altered perceptions of time and distance. Moderately larger doses of 10 mg or more produce effects more difficult to predict, such as muscle rigidity, lack of coordination, combative behavior, and auditory/visual hallucinations.

The results of PCP testing in fatally-injured drivers and DUID suspects over a 12-month period in Harris County, Texas. Attendees will learn about the pharmacology and behavioral effects of PCP, as well as modern analytical methods for determining concentrations in forensic specimens. Participants will also learn about the prevalence of PCP in arrested and deceased automobile drivers, and witness some of the characteristics of PCP-related MVA scenes.

In the deceased driver group, PCP blood levels were between 0.09 and 0.20 mg/L. No other drugs, except ethanol, (0.08 and 0.01 mg/dL in two cases) were detected. Typically, the vehicles left the roadway at a high speed, striking fixed objects without any evidence of skid marks or braking. In each of these cases, the collisions resulted in severe damage and almost appeared to be intentional. From a medical examiner viewpoint, this behavior may be interpreted as a suicide in contrast to an accidental collision.

In two of the DUID cases, PCP blood levels were determined to be 0.016 and 0.052 mg/L. In the remaining DUI cases, the presence of PCP was confirmed in urine since it was the only available specimen. Marijuana use was also detected in four of the DUID cases, which points toward the combinatorial use of marijuana and PCP, otherwise known as "fry." Two of these cases were also positive for cocaine and alprazolam.

These cases illuminate the extreme danger of driving under the influence of PCP and also provide support for increased vigilance in apprehending impaired drivers. Simple analyses for alcohol alone might not have been sufficient to interpret these cases satisfactorily. Due to the relatively long half-life of PCP in comparison to other drugs of abuse, the extended excretion interval allows sufficient time to find evidence of consumption that can be correlated to observed actions. Findings suggest that an expanded screen for common drugs of abuse can provide valuable information in the interpretation of motor vehicle fatalities and DRE evaluations.

Phencyclidine, Drivers, Accidents

K3 Comparison of Blood Alcohol Drink-Equivalent From Models and Breath Measurements

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After attending this presentation, attendees will learn about a comparison of blood alcohol drink-equivalent calculated from model equations of Widmark (1932), Watson et al. (1980), Forrest (1986), Urich et al. (1987) and Seidl et al. (2000) with that from breath alcohol testing.

This presentation will impact the forensic community by furthering validation of model calculations of alcohol concentrations applied to breath alcohol testing to assist qualifying some expert opinion that may utilize such calculations to assist in judicial processes.

Model calculations of alcohol concentration may have differing applicability to subjects within a demographic group involving gender, age, weight, height, and body mass index (BMI).

Test subjects (675 male and 100 female) were obtained from a forensic population of motor vehicle drivers under direction of their legal counsel. Informed consent excluded persons seeking, or having received, counseling and/or medical treatment concerning alcohol, and those with limiting physical or mental health. Some subjects consumed a light breakfast hours prior to their arrival for testing and initiating dosing from an alcohol-free state. After consumption of their commercial alcohol beverages (with identified concentration) over a median (with range) of 153 minutes (14–290), suitable breath samples from subjects in the elimination phase were analyzed about every 20 minutes for their alcohol concentration using either an Intoxilyzer 5000 (1 of 7 instruments; 622 subjects) or Breathalyzer 900/900A (1 of 10; 153 subjects). Volume and duration of subject breath samples were monitored concomitantly by spirometry for many cases with Intoxilyzer testing; duration was recorded for a Breathalyzer test and volume from another immediate sample. Instrument calibration was confirmed using forensic alcohol standards from different manufacturers with differing concentrations. Model calculations included weight per volume units using the density of blood (1.055 g/mL) for comparison with breath alcohol concentration (mg/210 dL). The "r" factor in Widmark for females (0.61) used combined results of Österlind et al. (1944). Separate analysis of Breathalyzer and Intoxilyzer data found no significant difference to their combination.

Subjects (male and female) had medians (with range) of: age (yr) 39 (17–77) and 37 (19–74); weight (kg) 81.6 (44.0–144.9) and 64.9 (41.0–132.0); height (cm) 176.5 (155.7–203.2) and 163.8 (149.8–187.1); and BMI of 26.3 (16.5–42.6) and 23.7 (16.2–48.4). Fifteen other persons were excluded for protocol non-compliance. The alcohol dose (g) per body weight (kg) had a median for males of 1.11 (0.58–2.24) and females of 0.98 (0.75–1.77) that generated maximum breath alcohol concentrations (mg/210 dL) with a median for males of 118 (46–216) and females of 124 (57–171). The median experimental alcohol drink-equivalent (mg/210 dL) for males was 24.8 (13.9–37.6) and females was 35.5 (17.8–59.2). Median variation in alcohol drink-equivalent (mg/210 dL) using equations of Widmark, Watson, Forrest, Ulrich and Seidl and breath alcohol testing were for males: 0.92 (-8.7–16.9), 0.30 (-7.7–9.1), 0.23 (-7.4–9.1), -0.64 (-8.7–7.3) and -1.1 (-9.4–7.6), and females: 0.89 (-9.8–13.9), 0.57 (-16.0–13.3), -0.83 (-16.1–13.0), not available, and -0.48 (-16.7–31.4). If the relative factor for females from only Österlind et al. was used (0.637/0.697), then the median variation was 0.20 (-10.7–13.2). Also found were: (1) higher variation (mg/210 dL) for females than males with all models; (2) Widmark for low body weight or BMI tend to overestimate concentration, and (3) Seidl for females with high BMI tend to overestimate concentration. The best fit to breath alcohol testing were calculations for males by Forrest, and females by Seidl. Both gender combined were best fit by Watson, then Forrest, then slightly less by Seidl and Widmark: no model exists for females by Ulrich.

Calculations of alcohol drink-equivalent using models (Widmark, Watson, Forrest, Ulrich, Seidl) agreed with that from breath alcohol testing for mean proportions of subjects at limits (± mg/210 dL) for males: 66.8% (62.1–70.4) at ±2.5, 92.7% (91.0–94.7) at ±5.0, and 98.9% (97.9–99.7) at ±7.5, and females: 65.8% (59.0–69.0) at ±4.5, 93.5% (90.0–97.0) at ±9.0, and 98.3% (97.0–99.0) at ±13.5.

Alcohol, Model, Concentration

K4 National, Regional, and Local Patterns of Methadone and Buprenorphine Seized by Law Enforcement and Analyzed by Crime Laboratories in the United States: 2003-2008

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After attending this presentation, attendees will better understand the trends and geographical variation of drug seizures that inform the U.S. Drug Enforcement Administration (DEA) of the trafficking and potential diversion of methadone and buprenorphine (generic names for two opioid analgesics).

The presentation will impact the forensic community by acknowledging the large contribution of crime laboratory forensic

scientists, as well as the importance of forensic laboratory data. The presentation will also contribute to a clearer understanding of varying dimensions and components of the trafficking, diversion, and abuse of methadone and buprenorphine.

The diversion and abuse of methadone is a key issue for United States drug control agencies, as is the expanding non-medical use of buprenorphine, an alternative to methadone treatment for heroin addiction. The heightened level of concern associated with these drugs is demonstrated in part by the frequency by which methadone and buprenorphine have been obtained by law enforcement agencies and analyzed by our Nation's crime laboratories over the past six years. Data from DEA's National Forensic Laboratory Information System (NFLIS) will be presented on methadone and buprenorphine, two synthetic opioid analgesics. NFLIS data represent instances where these drugs were seized by law enforcement and analyzed by forensic laboratories. From 2003 to 2008, the number of methadone and buprenorphine items reported by state and local laboratories increased significantly in the United States ($p<0.05$). Methadone more than doubled from 4,967 items in 2003 to 10,459 items in 2008, while buprenorphine significantly increased from 25 items in 2003 to 5,627 items in 2008.

Table 1. National and Regional Estimates for Methadone and Buprenorphine, 2003-2008.
Estimated number of total analyzed methadone and buprenorphine items, 2003-2008.

	TOTAL	2003	2004	2005	2006	2007	2008
Methadone							
National	49,308	4,967	6,397	7,302	9,822	10,361	10,459
West	7,066	546	802	1,074	1,280	1,611	1,753
Midwest	7,970	859	1,038	1,037	1,624	1,656	1,756
Northeast	12,200	1,526	1,988	1,847	2,488	2,241	2,110
South	22,073	2,036	2,569	3,345	4,431	4,852	4,840
Buprenorphine							
National	11,371	25	262	540	1,809	3,108	5,627
West	427	*	*	*	*	163	264
Midwest	985	*	*	*	127	282	576
Northeast	6,323	21	244	427	1,254	1,746	2,631
South	3,514	4	10	61	366	917	2,156

*The estimate for this drug does not meet standards of precision and reliability.

Highlighted findings will include the prevalence of methadone and buprenorphine items reporting to NFLIS at national, state, and local levels from 2003 to 2008. State and county-level maps will be used to display levels of seized drugs identified in the United States. The exploration of geographically specific information provides timely information on drug trafficking and abuse spatial patterns. This level of understanding is vital as the diversion of methadone presents an increasing threat to public health. For example, methadone-related deaths in the United States increased nearly 600% from 1999 to 2006. As buprenorphine is prescribed more for opioid dependence therapy, impacts on the nation's health may also ensue.

Methadone, Buprenorphine, Prescription Drugs

K5 The Analysis of Oral Swabs by F-SPE/ Fast LC-MSMS for Low Level THC

Jeffery Hackett, MSc, Northern Tier Research, 1300 Old Plank Road, Mayfield, PA 18433; and Albert A. Elian, MS*, 59 Horse Pond Road, Sudbury, MA 01776*

After attending this presentation, attendees will learn how useful oral swabs taken from living individuals can be for the analysis of tetrahydrocannabinol (THC). This situation may arise when other samples are limited or are unavailable. The data presented in this presentation should add another technique for THC analysis in facilities providing toxicological services.

This presentation will impact the forensic science community by demonstrating how useful F-SPE and LC-MS/MS in the confirmation/ quantification of low level THC in oral swabs.

Methods: Over 10 consecutive days, oral swabs were taken from a donor (who used THC) 1 hour after smoking. The swabs were individually air dried, packaged and submitted to NTR/MSPCL. The samples were extracted in a glass tube with 500 µL of methanol (containng THC-D3) by soaking for 30 minutes. Before removal from the tube, each swab was washed with a further 100 µL of methanol. Each sample was evaporated to approximately 200 µL before 5 mL of phosphate buffer (pH 7) was added. This solution was extracted by fluorous solid phase extraction (F-SPE). The columns were conditioned with methanol, deionized water, and pH 7 phosphate buffer (3 mL, 3 mL, and 1 mL, respectively). After washing with deionized water and pH 7 buffer (3 mL of each), the columns were dried and eluted with hexane: ethyl acetate (50:50 v/v) containing 2% acetic acid. The eluates were evaporated to dryness under nitrogen and reconstituted in 50 µL of mobile phase for analysis by fast LC-MSMS using 20µL for injection.

Chromatographic analysis was performed on a 50 x 2.0 mm (5 µm) C_{18} column, with a gradient program of acetonitrile and 0.1% aqueous formic acid that ran for 4.5 minutes. Tandem mass spectrometry was performed in postive and negative MRM mode (to screen for any THC-acid present)

THC / THC-acid calibrators were set up by extracting 0.25, 1, 2, 5, 10, and 50 ng/ mL, controls were set up at 4 and 15 ng/ mL from aqueous buffer samples (5 mL). From the analysis of the calbrators and controls: r^2 value> 0.995, recoveries > 85% were obtained. Limits of detection/ quantification of 0.1 and 0.25 ng/mL, respectively were acheived.

Result: Of the 10 oral swabs where oral swabs taken 1 hour after administration, 9 were found to be postive (THC). The levels of THC ranged from 0.5 ng to 2.5 ng/ mL. None of the swabs contained the THC-acid metabolite.

Conclusions: Based on data presented, the use of oral swabs may be used to extract, confirm, and quantify low levels of THC. The employment of both F-SPE and fast LC-MS/MS shows that this procedure can be performed efficiently and rapidly, which is to the benefit of all scientists in forensic toxicology.

THC, F-SPE, LC-MS/MS

K6 A Retrospective Comparison of Blood- and Breath- Alcohol Results in Wisconsin Impaired Drivers 2001-2007

Patrick M. Harding, BS, Wisconsin State Laboratory of Hygiene, Toxicology Section, PO Box 7996, Madison, WI 53707-7996; and Rod G. Gullberg, MPA, Washington State Patrol, 811 East Roanoke, Seattle, WA 98102*

After attending this presentation, attendees will better understand the relationship between breath- and blood-alcohol measurements and be better able to assess the validity of breath alcohol results as applied to a population of suspected impaired drivers.

This presentation will impact the forensic community by providing useful data by which to assess claims of breath alcohol testing unreliability as well as providing practical data relating the theoretical blood:breath alcohol ratio in the target population of impaired drivers.

Laws in most jurisdictions define illegal per se alcohol impaired driving offenses in terms of both breath and blood alcohol concentrations. Even so, the relationship between breath and blood alcohol results is still raised as an issue in court cases and a comparison of the two can yield insight into the prevalence of falsely elevated breath alcohol results, as is frequently alleged. In this retrospective study data is compared from drivers arrested for impaired driving offenses in the

State of Wisconsin who had both breath and blood alcohol specimens analyzed. Breath alcohol testing was conducted in the field on EC/IR and EC/IR II (Intoximeters, Inc., St. Louis, MO) breath alcohol analyzers. Breath alcohol results are obtained on duplicate breath samples and must agree with +/- 0.020 g/210L. The lower of the two three decimal place acceptable results is truncated to two decimal places as the reported result. Blood specimens were collected by medical personnel and submitted by the arresting agency to the Wisconsin State Laboratory of Hygiene (WSLH). Blood analysis was performed by direct injection gas chromatography, with 10% done in duplicate per the testing protocol in effect during the study period. All testing was done as part of the routine investigation of impaired driving cases. Only positive breath and blood alcohol results obtained within three hours of each other were included in this study.

During the study period of 2001-2007 there were 1,744 cases that met the inclusion criteria. Of these cases there were 1,545 males with a mean age of 34.9 (16-84) and 199 females with a mean age of 35.8 (16-77). The mean reported breath alcohol concentration (BrAC) was 0.144 g/210L (0.01 – 0.40). The mean of the un-truncated individual BrAC results was 0.1510 g/210L (0.010-0.400). The mean blood alcohol concentration (BAC) was 0.1624 g/100ml (0.010-0.450). The mean difference (BAC-BrAC) between reported BrAC and reported BAC results was 0.018 (-0.046-0.100). There were 869 cases where the BrAC was collected before the BAC with a mean elapsed time of 0.89 hours (0.13 – 2.75). There were 875 cases where the BAC was collected before the BrAC with a mean elapsed time of 0.80 hours and range of 0.05 to 2.27 hours.

BAC results were adjusted for the alcohol elimination occurring between the breath test time and the time of blood collection using a rate of 0.019 g/100ml per hour. After adjustment the BAC – BrAC differences were a mean of 0.019 (-0.029-0.082). The reported BrAC exceeded the adjusted BAC in 105 cases (6.0%). Of these, only 23 (1.3%) exceeded the BAC by 0.010 or more (range 0.010-0.029). There were only five cases where the BrAC was 0.08 and the adjusted BAC was below 0.080. Of these only one differed by more than 0.010. Blood:Breath alcohol ratios were calculated using the time-corrected BAC and reported BrAC. The mean (SD) ratio was 2428:1 (295) (range1622:1 – 6822:1.

The findings of this study are in agreement with others that have found that evidentiary BrAC results generally underestimate BAC in the driving population. The absence of significant overestimations of BAC by BrAC in this study provides strong evidence that alleged significant elevations of BRAC by mouth alcohol, GERD, potential interfering substances, variations in lung capacity, breathing disorders, etc. do not occur in the context of a well-regulated evidential breath testing program.

Forensic Toxicology, Blood Alcohol, Breath Alcohol

K7 Cocaine Detection in Postmortem Samples Following Therapeutic Administration

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After viewing this presentation, attendees will understand the importance of a thorough investigation, medical records review, and interpretation of autopsy and toxicology results in postmortem cases in which controlled substances such as cocaine are detected following administration in a clinical setting.

This presentation will impact the forensic science community by emphasizing that the detection of illicit drugs in postmortem samples is not always indicative of abuse.

Cocaine is a drug which is notorious for its high potential for recreational abuse, and the detection of cocaine in postmortem samples would most often lead toxicologists and forensic pathologists to believe that the drug was abused. However, cocaine is an effective local anesthetic and vasoconstrictor of mucous membranes and has been used clinically in surgeries of the eye, ear, nose, and throat for over 100 years. The persistent popularity of the clinical use of this drug is clearly attributable to its unique ability to simultaneously limit epistaxis and induce local anesthesia. Therefore, it is important to note that the presence of cocaine and its metabolites in postmortem samples cannot always be attributed to abuse and that a thorough investigation and review of clinical records is warranted before an informed conclusion can be made.

Presented here is the case of a 54-year-old male who was involved in an altercation during which he suffered multiple injuries. Three days later, a surgical procedure involving closed reduction of bilateral nasal bone fractures was performed and the man was released from the hospital. Approximately eleven hours post-surgery, the man was found unresponsive in bed and EMS responded and pronounced him dead on the scene. Given the circumstances leading up to the demise, a full postmortem examination was performed in order to elucidate the contribution of external factors such as physical injury, surgical intervention, and/or drug use to his death. In addition to natural disease and injuries documented at autopsy, toxicological analysis revealed the presence of cocaine metabolites in the man's urine. A comprehensive review of subsequently received surgical records revealed that the man was administered cocaine during the procedure to repair his nasal bone fractures.

If not for this review of surgical records, the assumption of cocaine abuse might have otherwise been made and the well-known cardiotoxic effects associated with cocaine considered a contributory factor in certification of cause and manner of death. Additionally, an erroneous presumption of illicit drug use may have significant implications in a legal setting and may cause family members of the decedent undue anguish. Toxicology results, investigative reports, clinical records, and pathologic findings must be collectively taken into consideration to ensure accurate explanations for the presence of cocaine, as well as other drugs that may be administered clinically, in postmortem samples.

Postmortem, Cocaine, Therapeutic

K8 Gastric Fentanyl Concentrations in Fentanyl-Related Deaths: A Study of 11 Cases

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After attending this presentation, attendees will have a greater understanding of the role that the analysis of gastric concentrations of fentanyl has in fentanyl-related deaths.

This presentation will impact the forensic community by providing toxicological data and insights on potential relationships between gastric fentanyl concentration, blood fentanyl concentration, route of administration, and cause and manner of death.

Given that fentanyl is a short acting and potent narcotic, there is potential risk for abuse and fatalities. Interpreting postmortem

toxicology in suspected narcotic overdoses, including fentanyl, can be difficult for medical examiners due to the variety of drug use and abuse and the development of tolerance in the user/abuser. The unfortunate "creative" abuses of the patch (including snorting, smoking and chewing) only complicate the issue. A strategy utilized in postmortem toxicological evaluation has been to analyze gastric contents for the amount of drug present to ascertain a potential route of administration and/or the cause and manner of death. One would expect that oral consumption would lead to higher levels of gastric concentrations in general. If that were so, would one be able to use the gastric concentrations to determine the route of administration (including inappropriate use of the transdermal patch) as well as the manner of death (intentional versus accidental overdose)? The purpose of this current study was to determine the gastric concentrations of fentanyl and norfentanyl in fentanyl-related deaths and to attempt to relate these levels with blood concentrations, route of administration, and cause and manner of death.

From January 2007 to June 2009, eleven fentanyl-related deaths in which gastric samples were available were identified through routine toxicology testing in the El Paso County Coroner's Office toxicology laboratory in Colorado Springs, Colorado. Routine toxicological testing was performed on all cases. Ethanol and related alcohols were detected using headspace Gas Chromatography/Flame Ionization Detection (GC/FID), urine was screened for drugs of abuse by ELISA and Gas Chromatography-Mass Spectrometry (GC/MS), and GC/MS was used to quantitate the blood and gastric contents after a liquid-liquid basic extraction.

The age of the decedents ranged from 23 to 60 years and consisted of three men and eight women. The blood concentration of fentanyl ranged from 2.1 to 30.7 µg/L (mean 17.5 µg/L) while the total gastric fentanyl concentration ranged from 2.9 to 432.4 µg (mean 85.1 µg). The analytical data for norfentanyl concentrations in the gastric samples were inconclusive as the samples calculated below the detection level of 5 µg/L. The cause of death was acute fentanyl intoxication in six out of eleven cases while five cases were ruled mixed drug overdoses. The manner of death was accidental in eight cases and undetermined in three cases. The route of administration was by transdermal patch in nine cases, oral (by chewing the patch) in one case, and unknown in one case.

In conclusion, there did not appear to be any correlations between the gastric and blood concentrations of fentanyl, the route of administration, or the cause and manner of death. It did not appear to be helpful to determine if the individual had intentionally or accidentally overdosed, nor did it provide insight into the route of administration (e.g. inappropriate use of the patch). Although the case with oral route of administration had the highest gastric concentration of fentanyl, the level was not impressively higher than the next highest concentration, where the route of administration was transdermal application. Additionally, the oral route of administration did not yield the highest total gastric fentanyl concentration. A limiting factor in our study was the small subject number. Perhaps a larger study (e.g., a multi-centered study) focusing on the analysis of gastric concentration of fentanyl would be useful and illuminate any useful patterns or trends.

Gastric Fentanyl, Fentanyl Overdose, Forensic Toxicology

K9 Exposure to Limonene: A Case Report

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After attending this presentation, attendees will learn the relation between orange oil (Limonene) and respiratory failure.

This presentation will impact the forensic community by providing the relation between orange oil (Limonene) and respiratory failure.

D-limonene (4-Isopropenyl-1-methylcyclohexene) is the chemical name for orange oil. It's a renewable resource and is a by-product of orange juice manufacturing. Orange oil is the oily substance found in the rinds of oranges. Orange oil is used in cleaning solutions, pet shampoos, soaps and perfumes. Limonene and its products are skin and respiratory irritants. Acute exposure to D-limonene has rarely been reported in deaths.

In this case report, we present a case of a previously healthy 30-year-old man who presented to the emergency department with acute respiratory failure. Non-toxicological causes were excluded. The purpose of this work was to demonstrate a toxicological cause of the respiratory failure and to recommend full toxicological screening for clinical and postmortem cases, especially those under suspicious circumstances.

A complete history was taken and a comprehensive clinical examination was performed. Toxicological analysis was performed. For the analysis of limonene in blood, gas chromatography/mass spectrometry (Shimadzu 2010) was utilized in the splitless mode of injection. The initial temperature was 170◦C for 2 min. and then programmed at 16◦C /min. to 270◦C and held for 8 minutes.

The assay was found to be linear in the concentration range of 0.5-20 ng/ml for limonene. Repeatability and intermediate precision were found to be less than 12% for all concentrations tested. Under standard chromatographic conditions the run cycle time would have been 13 minutes. By using fast chromatographic separation conditions, the assay analysis time could be reduced to 7 minutes without compromising the chromatographic resolution.

This developed procedure was also used to determine the limonene concentration levels for more than a hundred real forensic cases. The case was diagnosed as toxic exposure to limonene dissolved in organic solvent. The patient was exposed to limonene at home for many years in air fresheners. The patient survived after supportive treatment. The clinical and laboratory findings are discussed.

Limonene and its oxidation products are skin and respiratory irritants. Inhalation of these chemicals carries the risk of toxicity, which could be missed in diagnosis and hence treatment. This should encourage physicians working in emergency units to analysis for all available chemicals to avoid misdiagnosis.

Forensic, Chemical, GC/MS

K10 Study of the Effects of pH, Temperature, and Time on the Migration of Endocrine Disrupting Compounds From Polyethylene Terephthalate (PET) Bottles

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After attending this presentation, attendees will be informed of an important toxicological topic that has an effect on everyone. The attendees will also be exposed to new optimized extraction and analysis methods of several endocrine disrupting compounds which they can apply to further this aspect of work or use in their own current research projects.

This presentation will impact the forensic community by informing the audience of the constant presence of endocrine disrupting compounds (EDCs) and their possible effects on our health and environment. Although this work is only a start, it may lead to new safety measures to ensure that all levels of EDCs are below the oral reference dose. This work could have an effect on humanity because if these new measures were established, it could affect our everyday lives. Also, several extraction and analysis methods that are commonly used in forensic laboratories, have been used in this study. These methods include solid phase extraction (SPE) and gas chromatography-mass

spectrometry (GC-MS). This presentation may allow the audience to become more knowledgeable about these different techniques which they could possibly apply to their own work. Finally, a major challenge in this work was preventing external contamination. The clean techniques that were utilized in this study could also be useful in a forensic laboratory.

Several toxicological studies have shown that many common endocrine disrupting compounds (EDC), specifically those that display estrogenic properties, could cause toxicity from chronic exposure to levels as low as 20 μg/kg/day. Any compound that has the ability to alter hormonal homeostasis is considered to be an endocrine disrupting compound. Effects of exposure include abnormal cell growth, teratogenicity, liver injury, abnormal thyroid function, and reproductive toxicity. Two types of EDCs, alkylphenols and phthalates, have been found to migrate from plastic containers into the food supply. Recent studies have suggested that phthalates may have a cumulative effect, which has lead to a great interest in studying their presence in the environment. Two different extraction methods, solid phase extraction (SPE) and liquid liquid extraction (LLE), have been performed and compared. LLE has been found to be the most effective extraction technique for studying trace levels of EDCs in water. Gas chromatography- mass spectrometry (GC-MS) was chosen as the analysis method because of the reduced risk of contamination when compared with liquid chromatography- mass spectrometry (LC-MS). Many studies have suggested that LC-MS is not an efficient analysis method when studying EDCs, such as phthalates, because of the use of organic solvents and plastic tubing which can increase the risk of sample contamination. The specific compounds that are being studied include dimethyl phthalate (DMP), dimethyl terephthalate (DMT), diethyl phthalate (DEP), dibutyl phthalate (DBP), butyl benzyl phthalate (BBP), bis-(2-ethylhexyl) phthalate (DEHP), nonylphenol, and octylphenol. Several conditions were established in order to study the effects of temperature, plastic thickness, and pH on migration. When studying the effects of temperature, two plastic bottles were filled with drinking fountain water and were heated to 60°C for six hours. Samples were collected after every two hours. The second bottle had a thickness that was 50% less than the first bottle. Another bottle was filled with fountain water and stored at room temperature. This was used as a control for the temperature experiment and was sampled after three days. The effect of two different pH values was also studied. The two values used were 3.75 and 6.6. These values were chosen because they are comparable to different beverages that are commonly stored in plastic containers. The pH of each sample was adjusted by adding glacial acetic acid until the desired pH value was obtained. A third bottle was filled with fountain water and was not adjusted. This bottle was used as a control for the pH experiment and had a pH value of approximately 7.2. The three pH samples were collected after one day. When heated to 60°C for two hours, five of the eight compounds were detected. These compounds include DMP, DMT, DEP, OP, and DEHP, at concentrations of 2.144, 1.78, 1.258, 0.783, and 1.539 μg/L, respectively. These compounds were found in the control sample at concentrations of 0.619, 0.875, 0.18, 0.475, and 0.483 μg/L, respectively. Therefore, heating plastic to 60°C increased the amount of migration occurring. When the thickness of the plastic bottle was reduced by 50% these compounds were found at concentrations of 1.627, 0.979, 0.779, 0.601, and 1.562 μg/L, when heated at 60°C for two hours. Although lower concentrations were found to migrate from thinner plastics when heated, the migration occurred over a longer period of time when compared to the 50% thicker plastic. Migration was also found to increase as the pH of the water decreased, when stored at room temperature. All reported concentrations were determined using calibration curves prepared from standard solutions. Standard solutions containing all eight compounds were prepared at concentrations of 5×10^5, 5×10^4, 5×10^3, 500, 50, 25, 12.5, and 6.25 μg/L. These standard solutions were also used as controls in order to determine the retention times of each of the studied compounds. A selected ion monitoring (SIM) method was also developed and used to analyze trace levels of these compounds. When compared to a NIST library the highest matches obtained were 80% (DMP), 81% (DMT), 81% (DEP), 73% (OP), and 72% (DEHP). All identified compounds had approximately the same retention times as the standard solutions. The average concentration found from all experiments was 1.046 μg/L. When comparing to the previously reported value of 20 μg/kg/day, a 70 kg person would have to drink approximately 1339 L/day in order to be exposed to this toxic level. Overall, temperature, pH, and plastic thickness have been found to have an effect on EDC migration from PET bottles; however, all concentrations have been below any known toxic level. Further work will be performed in order to study the effect time may have on migration and to test the reproducibility of this method. It is important to understand the effects any of these factors may have on EDC migration because it may or may not suggest that certain safety measures need to be established in order to ensure that all levels are below the oral reference dose (RfD) values set by the United States Environmental Protection Agency.

Toxicology, Endocrine Disrupting Compounds, Gas Chromatography-Mass Spectrometry

K11 Mixed Prescription Drug Death

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After attending this presentation, attendees will become aware of the effects of mixing prescription drugs.

This presentation will impact the forensic community by increasing the awareness of the role of prescription drugs in deaths.

This laboratory has been detecting an increase in the prevalence of multiple prescribed opioid compounds in drug related postmortem cases. In most of our cases, the decedents have histories of addiction to oxycontin and xanax.

A case of postmortem analysis will be presented of a 36-year-old single white male who consumed Oxycontin, Xanax, and Actiq (fentanyl lollipop). The decedent had morbid obesity (BMI of 46) with a long history of lower back pain for at least six years. His clinical work-up for his lower back pain was inconclusive, although a spinal surgery for disc fusion was suggested at one point. He was known to visit various pain clinics and acquired prescription Actiq for at least 18 months. According to multiple co-workers, he demonstrated a typical pattern of opioid compound addition which interfered with his job performance significantly. During his last year, he was admitted twice at a local rehabilitation center for his addition to Oxycontin and Xanax. Of note, per multiple co-workers and family members, the decedent had no prior suicidal ideation or attempt.

The decedent was discovered in his residence with early decomposition after failing to report to work as an accountant. Scene investigation found a fentanyl lollipop inside his shirt pocket as well as crushed pill fragments scattered on the floor and table. Multiple bottles of prescription oxycodone and alprazolam, some empty and others near-full, were located at the scene. Autopsy revealed hypertensive cardiovascular disease and focal bronchopneumonia, and both were not considered to be medically significant. Comprehensive analysis was performed on various postmortem tissues, including femoral blood, urine, stomach content and liver.

All submitted tissues were subject to standard analytical screening and mass spectrometry confirmation protocols. Positive findings of the analysis are as follows:

Blood		
	Oxycodone	1.86 mg/L
	Oxymorphone	0.072 mg/L
	Fentanyl	64 mcg/L
Liver		
	Oxycodone	1.83 mg/Kg
	Oxymorphone	0.12 mg/Kg
	Fentanyl	96 mcg/Kg
Stomach Contents		
	Total weight received: 244 gms	
	Oxycodone	147.30 mg/Kg
	Oxymorphone	0.62 mg/Kg
	Fentanyl	3648 mcg/Kg
Urine		
	Oxycodone	5.82 mg/L
	Oxymorphone	0.36 mg/L
	Fentanyl	890 mcg/L
	a-hydroxyalprazolam	1.60 mg/L
	Alprazolam	0.35 mg/L

Note: Alprazolam and its metabolite were only detected in the urine specimen.

The medical examiner ruled the cause as multiple drug intoxication and manner as accidental.

Oxycodone, Fentanyl, Prescription

K12 Analytical Method Development for Determining the Biomarker, 2-Aminothiazoline-4-Carboxylic Acid (ATCA), in Mice Liver After Cyanide Exposure

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After attending the presentation, attendees will learn about new methods of detecting cyanide exposure and the analytical techniques that are being developed to test the presence in mice livers. The attendees will also learn about 2-Aminothiazoline-4-Carboxylic Acid (ATCA) as a biomarker for cyanide.

This presentation will impact the forensic science community by demonstrating a forensic application being developed to detect cyanide poisoning postmortem. This research will be able to be applied to human remains that are in autopsy under investigation for poisoning. ATCA is a stable biomarker for cyanide so this technique will be able to be applied to cold cases.

The objective of this research was to develop a new analytical technique to determine the chemically stable urinary metabolite of cyanide, 2-aminothiazoline-4-carboxylic acid (ATCA), in mice liver samples. Two extraction techniques, solid phase extraction (SPE) cation exchange and molecular imprinted polymer stir bar (MIP-SB), were tested to determine the efficiency of ATCA extraction from mice liver samples. Mice were exposed to different doses of cyanide, and a method was developed to dissect, preserve organs, and homogenize the livers.

This research will be able to be applied to human remains that are in autopsy under investigation for poisoning. ATCA is a stable biomarker for cyanide so this technique will be able to be applied to cold cases. For forensic casework, a stable and quantifiable marker is needed to determine an accurate level of exposure postmortem. This method will be able to be used in cold cases because ATCA is a stable metabolite that stays in the body after the initial dose of cyanide is depleted.

Endogenous ATCA is always present in the body in low quantity originated from dietary intake of cyanide, smoking, fires or the normal metabolism of amino acids. A selective and sensitive analytical method is needed to determine the endogenous level of ATCA or identify cyanide poisoning. The use of ATCA as a biomarker for cyanide poisoning is promising due to its stability at ambient, as well as freezing temperatures and its production is directly related to cyanide exposure.

The molecularly imprinted polymers (MIPs) are made on the surface of a silica cylinder to serve as a selective stir bar sorption extraction (MISBSE) device. From an external calibration, the capacity of one MISBSE for ATCA was about 31 ng. The data showed that 700 rpm was the optimum stir speed and that sorption plateau was reached after 30 minutes of extraction time. Under the optimal extraction conditions, the MISBSE could selectively extract ATCA from urine samples. The MISBSE has improved the ability to extract lower concentrations of ATCA. Combining MISBSE with Liquid Chromatography Mass Spectrometry (LC/MS/MS), ATCA was detected without the use of any derivatization process. The solid phase extraction cation exchange was preformed with Oasis® MCX (mixed-mode cation exchange) columns and underwent several washes to prepare the cartridges for absorbing the ATCA and then was eluted with ammonium hydroxide with the assistance of a vacuum pump.

An effective method of preparing liver samples from the cyanide exposed mice for extraction will be presented. In additions, the two extraction methods (SPE *vs*. MIP-SB) will be compared. The effectiveness of the extraction techniques will be determined by employing known concentrations of ATCA evaluated by the LC/MS/MS. Liver ATCA contents will be compared to the dose of cyanide mice were given. This new analytical method may serve as great potential benefits for the toxicology field and forensics in general.

Molecularly Imprinted Polymer (MIP), 2-aminothiazoline-4-carboxylic acid (ATCA), Cyanide

K13 Pesticide Intoxications in Cukurova, Turkey: A Three Year Analysis

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The goal of this study is to show the distribution of pesticides in the Cukurova region and alert the forensic toxicologists to notice pesticides in all autopsy cases at this region.

This presentation will impact the forensic science community by demonstrating how organochlorine pesticides are still a serious threat for public health although the WHO has forbidden the use of these substances all over the world.

Cukurova region is one of the most important agricultural areas for Turkey. As a consequence of wide pesticide use, acute pesticide poisoning cases are quite common, in this region. These poisonings are generally suicidal self poisonings, while can be accidental or homicidal as well. In Cukurova, pesticide poisonings still remain as a considerable cause of death, which lead the present retrospective evaluation.

The autopsy records of Adana Group Authority of the Council of Forensic Medicine, between 2006 and 2008, were evaluated retrospectively. Deaths that are attributed to pesticide poisoning included in the scope of the study in order to identify the type of pesticide, as well as the etiology. The frequency and distribution of intoxications were also analyzed in terms of sex and age.

In the studied period, a total of 4,199 autopsies had referred to the forensic toxicology laboratory for pesticide analysis. Pesticide analyses were performed in the Forensic Toxicology Laboratory of Adana Group Authority of the Council of Forensic Medicine, using different biological samples (blood, stomach, liver, lung, and kidney) by chromatographic methods, gas chromatography with electron capture detection (GC-ECD), and gas chromatography with nitrogen phosphorus detection (GC-NPD) and gas chromatography-mass spectrometry (GC-MS).

Seventy-two out of all cases were positive for pesticide analysis. Of these 72 cases, 42 (58.33%) were male and 30 (41.66%) were female, with a mean age of 38.8 ± 20.6 years. Among the inspected pesticides, endosulfan was found to be the most common with 47.2% prevalence, followed by an organophosphorus insecticide dichlorvos with a prevalence of 16.7%. Majority of deaths due to pesticide poisonings (37, 51.38%) were suicidal while (17, 23.61%) of them were accidental. The high ratio of suicidal deaths due to pesticides was a consequence of easy availability and accessibility of uncontrolled pesticides in households at city centers and in villages of countryside.

This report showed that endosulfan, an organochlorine pesticide, is commonly used in Cukurova region. Moreover, frequency of acute and chronic exposure to endosulfan is considerably high in Cukurova region. Recently, strict regulations have been enacted for restricting and controlling the use of endosulfan, of which use was previously allowed. Furthermore, authorities should set more efficient educational facilities for agricultural workers in order to reduce the number of accidental pesticide poisonings.

Forensic Toxicology, Pesticide Poisoning, Cukurova (ADANA)

K14 Identification of GHB and Morphine in Hair in a Case of Drug Facilitated Sexual Assault

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After attending this presentation, attendees will appreciate the importance of an accurate toxicological analysis in sexual assault cases.

This presentation will impact the forensic science community by detailing a unique circumstance of sexual assault and the drug GHB Gamma-hydroxybutyric acid, a substance naturally present in mammalian species, which has been utilized to commit the crime.

Gamma-hydroxybutyric acid GHB is qualified as a "predatory drug." Doses of 10 mg/kg cause amnesia, 20-30 mg/kg induce sleep and doses of 50 mg/kg or higher produce anesthesia. It is attractive for rapists because it can be found easily (on the street, fitness centers, and internet) and moreover because it can be delivered mainly as an odorless, colorless liquid and so it is often assumed unwittingly, mixed in spiked drinks.

Case Report: The case of a 24-year-old girl who was sexually assaulted after administration of Gamma-hydroxybutyric acid (GHB) and morphine will be presented. She had been living in an international college for foreign students for about one year and often complained of a general unhealthy feeling in the morning. At the end of the college period she returned to Italy and received at home some video clips shot by a mobile phone camera. In these videos she was having sex with a boy she met when she was studying abroad.

Materials and Methods: Toxicological analysis of the victim's hair was done: the hair was 20 cm long. A 2 cm segmentation of all the length of the hair was performed. Morphine and GHB were detected in hair segments related to the period of time she was abroad. The analyses of hair segments were performed by gas chromatography/mass spectrometry (GC/MS) and the concentration of morphine and GHB were calculated.

Conclusions: A higher value of GHB was revealed in the period of the criminal event and the presence of morphine was also detected for the same period. According to previous observations our case shows that hair analysis is the only method used to prove repetitive exposure to a toxic substance. This case demonstrates also that a high concentration of GHB in hair reflects an acute overexposure to GHB and can be documented several months after the sexual assault. In general it must be specified that the possibility given by hair analysis should not prevent the victim and the medical examiner from taking urine, blood, and sweat samples as soon as possible after the event. Hair analysis may be a useful adjunct to conventional drug testing in sexual assaults and it should not be considered an alternative to urine analysis, but a complement. It is possible that hair analysis could be a useful addition to conventional drug testing in sexual assault, but it is believed that further studies may confirm the usefulness of this technique and establish the definition of legally defensible cut-off values.

GHB, Hair Analysis- GC/MS, Drug Facilitated Sexual Assault

K15 Death by Potassium Chloride Intravenous Injection and Analytical Detection

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After attending this presentation, attendees will understand the results of the determination of blood potassium in a case of suicide by potassium poisoning. The meaning of the blood potassium concentration is questioned and discussed.

This presentation will impact the forensic science community by demonstrating how potassium concentration resulted to be significantly higher in heart blood in a case of suicide by potassium chloride intravenous injection and, therefore, the general issue of considering potassium poisoning hardly demonstrable by the toxicology needs to be questioned and thoroughly studied in the future.

Potassium chloride intravenous injection is reported as a means in suicide attempts and also in lethal procedures for state-sanctioned capital punishment. Owing to its relatively high concentrations in hemolyzed blood (25-80 mmol/l) as compared to serum (about 4 mmol/l), potassium poisoning has often been considered hardly detectable in postmortem blood specimens.

In considerations of the results of the determination of blood potassium in a case of suicide by potassium poisoning, the meaning of blood potassium concentration is questioned and discussed.

A 41-year-old man, working as a nurse at the local intensive care unit, was found dead at his workplace. A recent injection site was observed on his left foot and a syringe retrieved close to the corpse. At the autopsy no particular signs were noted.

Biological specimens (blood, bile, and urine) were submitted to the screening procedures for drugs and poisons in use in the laboratory, consisting of general unknown screening by solid phase extraction and gas chromatography mass spectrometry for blood and bile, head-space gas chromatography for blood and immunoenzymatic screening for urine. The syringe content was submitted to Feigl spot tests for inorganic ions and, in particular, for potassium. Finally, blood potassium concentration was determined by ion selective electrode measurement (linear over the range 3.0-150 mmol/l).

According to the routine screening procedure, blood was found positive for diazepam at therapeutic level (0.21 mg/l) and urine resulted positive only for benzodiazepines. No other substances were identified in blood and urine and all other samples tested negative. Potassium concentration was found at 160.0 mmol/l in cardiac blood and 87.3 mmol/l in femoral blood (mean of three determinations in both cases). On the other hand, hemolized blood samples obtained from autopsies with no relevant toxicological findings had much lower potassium concentration, i.e., between 32.2 and 43.0 mmol/l (median: 38.6 mmol/l, n=6).

Death by potassium intravenous injection is often considered undetectable by toxicological analyses when only hemolized blood is available, and, consequently, literature is relatively scant. These result show that potassium concentrations were significantly higher in heart blood in a case of suicide by potassium chloride intravenous injection and, therefore, the general issue of considering potassium poisoning hardly demonstrable by the toxicology testing needs to be questioned and thoroughly studied in the future.

Potassium Chloride, Intravenous Injection, Suicide

K16 A HPLC/MS/MS Method for Simultaneous Determination of Three Opiates and Three Benzodiazepines in Postmortem Blood

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After attending this presentation attendees will have new knowledge of a Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method for simultaneous determinations of morphine, hydromorphone, hydrocodone, alprazolam, diazepam, and nordiazepam in postmortem blood using multiple reaction monitoring (MRM) techniques and corresponding deuterated internal standards. Simultaneous analysis of multiple opiates and benzodiazepines has not previously been reported in postmortem blood.

This presentation will impact the forensic community by demonstrating an additional application of LC/MS/MS to the analysis of complex drug mixtures in postmortem specimens.

Analyses were performed with an ABI 3200 Q-Trap instrument operating in a positive polarity mode. For these analyses, one mL specimens of blood were basified by addition of saturated sodium borate buffer, and then extracted once with four volumes of the mixture of 1-chlorobutane and 2-propanol. The organic layer was evaporated and the residue was reconstituted into 0.5 mL of reconstitution solvent (aqueous buffer/acetonitrile; 9:1). Reconstitution solutions were filtered and chromatographed in an acetonitrile/ammonium formate gradient. Instrument parameters were optimized by infusion of solutions of each drug. The chromatographic column was maintained at 25˚C and the run time was 12.5 minutes.

The method was validated by examining selectivity, precision, accuracy, linearity, recovery, suppression, and limits of quantitation and detection. Calibration curves were quadratic for all analytes over the concentration range 10–1000 ng/mL, and correlation coefficients (R^2) were better than 0.999. Intraday and interday precision for all analytes at concentrations of 50, 200, and 500 ng/mL was between 4.1% and 10.6%, intraday and interday accuracy for all analytes at the three concentration levels was between 88% to 114%. Recoveries were between 13% and 52%. Limits of detection and quantitation were 3 and 10 ng/mL, respectively. Selectivity results demonstrate that the precision and accuracy of the analytes were not affected by the presence of 14 other common drugs. Only diazepam showed ion suppression in postmortem blood, and morphine and hydromorphone showed ion enhancement. Four postmortem blood specimens were analyzed by this method. The four specimens were also analyzed by alternate, individual LC/MS/MS opiate, and benzodiazepine methods. The results obtained by this new combined opiate/benzodiazepine method match well with the results run by the individual methods.

A method is described that is applicable to simultaneous determination of at least six opiates and benzodiazepines over a broad range of concentrations. Individual analytes were well separated, suggesting that the method is amenable to addition of other opiates or benzodiazepines.

LC/MS/MS, Benzodiazepines, Opiates

K17 Covalent Protein Adduction by Drugs of Abuse

Kevin J. Schneider, BS, 900 Euclid Avenue, #22, Miami Beach, FL 33139; Anthony P. DeCaprio, PhD, 11200 Southwest 8th Street, Miami, FL 33174*

After attending this presentation, attendees will glean some of the principles of covalent protein adduct formation, previous advances in generating and detecting protein adducts, how drugs of abuse form protein adducts, and the potential importance of protein adducts in the fields of clinical and forensic toxicology.

This presentation will impact the forensic science community by opening a new subset of toxicological analysis for clinical and forensic inquiries. It will allow for the development of new biomarkers of exposure to detect the use of addictive and illegal substances even after the parent compounds and major metabolites have been eliminated from biological samples. These persistent adducts can be employed to expand the range of time a substance can be detected in biological samples; a beneficial advance for both clinical and forensic applications.

Introduction: Protein adducts are formed by the covalent binding of an electrophilic, metabolically activated xenobiotic to nucleophilic sites on endogenous proteins or protein precursors. These permanent covalent bonds remain for the lifetime of the protein. However, not all nucleophilic sites on a given protein are equally reactive to activated xenobiotics. Certain amino acid residues are more susceptible to electrophilic attack, due to both steric and electronic factors. Each xenobiotic will interact with a given protein in a characteristic way determined by the combination of these factors.

While only a few studies have examined protein adduction by drugs of abuse, results suggest that this is a valuable issue to explore further. In this report, initial data is presented on such adducts by evaluating the relative binding affinity of several common drugs of abuse to amino acids known to be reactive under biological conditions. Initial research using a controlled *in vitro* exposure system is required prior to expanding studies into complex matrices and case studies involving biological samples from drug users. This initial step is germane to the understanding of how and where these drugs of abuse covalently bind to endogenous proteins and is imperative to the understanding of *in vivo* formation of protein adducts. This approach involved utilizing HPLC-MS, which allows for the sensitive and discriminating analysis of adducted peptide structure.

Methods: In order to generate protein adducts with drugs of abuse, an *in vitro* metabolic system was used. This system has previously been employed to assess hepatic metabolism of xenobiotics under controlled conditions. The system consisted of purified human cytochrome P450 3A4, human cytochrome b5, and human NADPH cytochrome P450 reductase in conjunction with required lipid cofactors (1,2-dilauroyl-sn-glycero-3-phosphocholine, 1,2-dioleoyl-sn-glycero-3-phosphocholine, and 1,2-dilauroyl-sn-glycero-3-phospho-L-serine). To each mixture, one of three test peptides was added: Ac-PAAHAA-OH, Ac-PAAKAA-OH, and Ac-PAACAA-OH. These short peptides allowed for the analysis of modifications of the reactive amino acid residue (His, Lys, and Cys,

respectively). Each peptide was tested with each of three common substances of abuse: cocaine, morphine, and methamphetamine (each at 200 μM final xenobiotic concentration). Peptides were incubated at 37°C in 200 mM phosphate buffered saline (pH 7.4) for 15 - 60 min to allow for metabolic activation of the xenobiotics and adduction to the peptides. Following incubation, 10 μL aliquots of the mixtures were acidified with TFA and introduced into a Varian 1000 LC-ion trap MS equipped with Polaris C18 column and optimized for the analysis of the individual peptides.

Results: Stable, time-dependent covalent adduction of all three model peptides at varying efficiencies was noted for each drug. Adduct formation was confirmed by the appearance of new peptide peaks with MS molecular ion and fragmentation data consistent with covalently bound drug. MS/MS with *de novo* peptide sequencing results confirmed the location of adduct at the putative reactive site for each peptide. Additional MS studies are currently ongoing to identify the reactive metabolite of each drug and the chemical structure of peptide-bound drug moiety. Data will be presented detailing the relative binding affinities for each tested drug of abuse and peptide.

Conclusions: The data presented in this study demonstrates the ability of metabolically activated cocaine, morphine, and methamphetamine to form viable adducts with nucleophilic residues of model peptides. The analytical detection of these adducts in *in vitro* studies provides the groundwork for further studies of *in vivo* production and analytical detection of these adducts in biological specimens.

Protein, Adduct, Toxicology

K18 Lethal Tachycardia Following a Low Dose of Clozapine

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The goal of this presentation is to report a case of lethal tachycardia that developed in a 99-year-old woman, who mistakenly received a low dose (50 mg) of clozapine.

This presentation will impact the forensic science community by showing how fatal cardiovascular side effect reported in association with clozapine may occur in very elderly patients even at low doses.

Clozapine is a well-proven antipsychotic agent with a wide atypical receptor profile. It is effective against both positive and negative symptoms, with sympatholytic, anticholinergic and antiserotoninergic side effects. It is particularly useful for the management of patients with schizophrenia who are either unresponsive to or intolerant of conventional antipsychotic agent. Use of clozapine is restricted to patients with treatment-refractory schizophrenia because of the drug's association with agranulocytosis, seen in about 1% of patients in the first year of treatment. Additional side effects that may occur with clozapine treatment include sleepiness, dizziness, seizures, pulmonary embolism and respiratory depression.

Recently, attention has focused on cardiovascular complications reported in association with clozapine. Cardiovascular side effects have been less commonly reported but have included orthostatic hypotension, tachycardia, electrocardiogram changes, myocarditis, and cardiomyopathy, which in some cases have resulted in the death of young people with no prior cardiac history.

Clozapine increases heart rate in the majority of patients and around 25% of individuals on therapeutic doses develop a mean increase of 10-15 bpm. Seemingly, the main causes are anticholinergic vagal inhibition and an increase in circulating catecholamines caused by α-1 adrenergic blockade.

Major symptoms in severe clozapine overdose are altered states of consciousness, agitation, confusion, delirium, coma, convulsions, tachycardia, arrhythmias and respiratory depression.

A case will be presented of lethal tachycardia following a low dose (50 mg) clozapine administration, occurred in a 99-year-old woman, after a nurse mistakenly gave her another patient's drug.

Thirty minutes after the administration, the patient was conscious and vital signs were normal. Blood pressure was 118/61 mmHg, heart rate was 85 bpm. One hour later, the patient was still conscious, blood pressure was 108/58, and heart rate was 85 bpm. One hour later, blood pressure was 171/101, heart rate was 101 bpm. Because of deterioration in her spontaneous respiration, the patient was endotracheally intubated and artificial respiration was applied. Gastric lavage could not be performed. All attempts to reanimate the patient did not lead to the clinical improvement and she died due to cardiac arrest after three hours of intensive care. An autopsy was performed at the University Center of Legal Medicine in Geneva. External examination was unremarkable. Internal examination showed congestion of internal organs and pulmonary oedema. Neuropathological investigation was negative. Histological examination showed moderate generalized congestion and broncho-aspiration of foreign material. Toxicological tests included blood ethanol levels and screening for common drugs and illegal substances by gas chromatography and mass spectrometry. Toxicological analysis showed midazolam (blood concentration 40 μg/l), which was administered to the patient at the hospital, and clozapine (blood concentration 140 μg/l), whose level was consistent with the administration of a 50 mg dose of clozapine.

Conclusion: The cause of death was determined to be clozapine intoxication

Adverse Drug Reaction, Clozapine, Tachycardia

K19 Preparation of Oral Fluid for Quantitative Determination of Opiates and Amphetamines by LC-MSMS

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After attending this presentation, attendees will better understand critical issues related to the analysis and quantitation of drugs of abuse in oral fluid.

This presentation will impact the forensic sciences community by expanding the current knowledge on issues related to the analysis of drugs in oral fluid and the distribution of drugs in oral fluid.

Keeping in mind that: (a) substitution therapy policy has recently been implemented in Taiwan; and, (b) oral fluid as an alternate specimen for monitoring drug use has attracted considerable interest, this project was carried out to develop a sample preparation method for effective analysis of opiates and amphetamines in oral fluid by the liquid chromatography-tandem mass spectrometry (LC-MSMS) technology.

Various heating and deproteinization parameters were evaluated for their effectiveness in: (a) removing forth, contaminations, and protein; (b) preserving original drug composition in the specimen; and, (c) carrying out direct electrospray LC-MSMS analysis. Oral fluid specimens were first processed by the sample preparation protocol, then analyzed by a LC-MSMS system (Agilent 6410 Triple Quadrupole Mass Spectrometer with an electrospray interface and an Agilent 1200 RRLC System) using an Agilent Zorbax SB-Aq (2.1 mm ´ 150 mm, 3.5 μm

particle) analytical column operated at 40 °C. The mobile phases adapted for gradient elution are: (A) methanol and (B) 0.1% (v/v) formic acid in water.

The established protocol achieved 1 ng/mL as the method's limit of detection for amphetamine, methamphetamine, 6-acetylmorphine, 6-acetylcodeine, morphine, and codeine. The method's limit of quantitation was 1 ng/mL for the first four compounds listed above and 2.5 ng/mL for morphine and codeine. The method was also successfully applied to the analysis of 34 oral fluid specimens collected from patients participating in the substitution therapy program following the institution's IRB guidelines. Data generating by the "sample preparation/direct LC-MSMS" protocol were superior to those obtained by portable testing devices and gas chromatography-mass spectrometry approaches. For example, one portable testing device could only identify 3 amphetamines and 1 opiates positives, while this method hereby developed quantitated the presence of methamphetamine, amphetamine, morphine, 6-acetylmorphine, and codeine in 20, 17, 7, 3, and 1 specimens. With the limited size of specimen available, the GC-MS approach could not detect the presence of the drugs of interest in many of the specimens that were found (by the newly developed methodology) to contain these dugs at low ng/mL concentration levels.

Oral Fluid, Drugs of Abuse, Liquid Chromatography-Tandem Mass Spectrometry

K20 Changed Contrast Agent Like Imagopaque the Concentration CNS Active Drugs by Cadavers?

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After attending this presentation, attendees will understand the basics of the downspout by a computed tomography angiography (CTA) and the effects from the contrast agent imagopaque on the concentration of the analyzed CNS active drugs.

After reading this poster presentation, the observer will understand the basics of the downspout by a computed tomography angiography (CTA) and the effects from the contrast agent imagopaque on the concentration of CNS active drugs to be analyzed. At the Centre of Forensic Imaging and Virtopsy in Bern, 22 selected decedents underwent a whole body computed tomography angiography (CTA). The cases were from 9 casualties, 10 natural deaths, 1 homicide, and 1 error in medical treatment. The gender of the cases were 7 women and 15 men. The mean age at time of death was 48.2 years, ranging from 3 to 85 years. The mean interval between estimated times of death and imaging was 29.5 hours, ranging from 5.5 to 70 hours.

A conventional autopsy, which started in the 5 hours later (mean) was performed in every case for a direct comparison with the radiologic findings. In all cases a mixture of a water-soluble, hydrophilic medium with polyethylene glycol (PEG) as a large molecular carrier substance and iohexol as the contrast agent in a mix ratio of 15:1 was used. During the angiography, we needed between 12.5 and 78.9 ml/kg KG from the contrast agent. Prior to the CT angiography, 10 ml peripheral venous blood was sampled from the femoral vein from each cadaver. On the occasion of the autopsy, the second venous blood sample was taken. In this study a comprehensive screening for central nervous system (CNS) active drugs was performed by LC/MS/MS. All analysis were carried out using an 1100 LC system (binary pump and autosampler) coupled to an API 4000 mass spectrometer equipped with a Turbo-Ion Spray source. The instrument software Analyst (ver. 1.4.2) was used for data processing. The multi target screening strategy is in principal described at Thieme & Sachs (2003).[1]

In five of the cases opioids, antidepressants, and benzodiazepines were detected in therapeutic ranges. The results of this study shows, that there are no new volumes of distribution and that the applied analytical method is practical. The most important result of the study is that a qualitative and quantitative analysis for drugs could be performed after a CTA with injected contrast volume.

Reference:

1. Thieme D, Sachs H, Improved Screening capabilities in forensic toxicology by application of liquid chromatography-tandem mass spectrometry. Anal Chim Acta 483,171-186, 2003

CTA, Drug Concentration, Toxicological Analysis

K21 Death Caused by Fentanyl Smoking

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After attending this presentation, attendees will have greater awareness that smoking should be considered as a potential route of administration in cases of fatal fentanyl intoxication.

This presentation will impact the forensic science community by informing attendees of an uncommon route of administration for a drug that has become increasingly important in drug overdose deaths.

Fentanyl is a highly efficacious synthetic opioid analgesic marketed under a variety of proprietary names. Fentanyl is commonly implicated in deaths attributed to recreational drug abuse in West Virginia; a state which currently endures the highest per capita rate of opioid fatality in the U.S. Fentanyl is clinically employed in combination with other analgesics for post-surgical pain control as well as in the treatment of chronic pain. When employed for outpatient chronic pain control, fentanyl is characteristically prescribed as a transdermal patch preparation. It is in this form that fentanyl is often diverted and fatally abused, with patch mastication and/or ingestion as well-described patterns of drug misuse. A case will be reported of fatal recreational fentanyl abuse by a method of drug intake rarely described in forensic literature: smoking of fentanyl patch material.

Authorities were notified when two unresponsive individuals were discovered in a parked car. The man in the driver's seat was pronounced dead on the scene. The investigating officer noted a piece of singed aluminum foil in the decedent's lap. The foil was retained as evidence and the decedent was transported to the medical examiner's office.

The body was that of a 33-year-old white male with a height of 74.5 inches and weight of 223 lbs. A complete autopsy revealed marked pulmonary edema but no apparent anatomical cause of death. Review of the decedent's medical records failed to demonstrate current or prior prescription access to fentanyl or other opioid pharmaceuticals. Specimens submitted for toxicological analysis included subclavian blood, gastric contents, urine, liver, and vitreous humor.

The following tests were performed by the toxicology laboratory: blood alcohol by GC-FID, blood precipitate immunoassay for drugs of abuse (EMIT), and acid/neutral and alkaline drug screens of blood by GC-MS. No alcohol or drugs were detected by these methods. A directed assay was then performed for fentanyl using liquid-liquid extraction of subclavian blood. Analysis was performed on an HP 1100 series LC-MSD using a Zorbax SB-CN column, APCI+ ionization, and data collection in SIM mode.

Fentanyl was identified and quantitated in the blood at 9.3 ng/mL, a concentration at which fatal toxicity has been reported. Norfentanyl, an

active metabolite, was not detected suggesting rapid accumulation of lethal fentanyl blood levels and respiratory arrest. No commercial fentanyl preparations were present in the vehicle where the decedent was discovered; however, the piece of aluminum foil was analyzed and found to be positive for fentanyl with no other drugs detected. Cause of death, as determined by peer review, was the result of non-prescribed fentanyl abuse and the manner was accidental.

The majority of fatal overdoses involving fentanyl use in West Virginia occur in the setting of multiple drug toxicity. This case of fatal fentanyl intoxication in a 33-year-old man was unusual in that no other drugs or alcohol were detected and that smoking was concluded to be the route of administration.

Fentanyl, Smoking, Postmortem

K22 Methcathinone Formation During Analysis of Ephedrine or Pseudoephedrine

Wendy R. Adams, PhD, Joseph Homan, MS, and Joseph Corvo, BS, NMS Labs, Inc., 3701 Welsh Road, Willow Grove, PA 19090*

After attending this presentation, attendees will gain awareness of how analytical artifacts can lead to false positive results, as illustrated by the formation of a schedule I substance from over-the-counter cold medicines present in blood and urine specimens. Suggested methods for detecting and avoiding this artifact will also be provided.

This presentation will impact the forensic science community by raising awareness of the potential for controlled substances to form as artifacts in specimens containing high concentrations of ephedrine or pseudoephedrine. The *in vitro* formation of methamphetamine has previously resulted in false positive reports on proficiency tests. Now it appears that methcathinone may also be formed *in vitro* with similar specimens and analytical techniques. Determining the source of the methcathinone in biological samples is essential for correct interpretation in postmortem and DWI investigations.

Methcathinone is a schedule I controlled substance easily synthesized by oxidation of ephedrine or pseudoephedrine. Use of methcathinone peaked briefly in the 1990s, but has since declined; in large part due to stricter control of pseudoephedrine. Methcathinone produces euphoric and stimulant effects similar to, but less intense than, methamphetamine. Methcathinone is not nearly as popular as methamphetamine, but is easier to synthesize, and may serve as a starting point for clandestine chemists. A recent raid in Valdez, AK uncovered a methcathinone lab in the home of an 18-year-old and a 16-year-old was arrested in Irvine, CA for experimenting with a methcathinone recipe she found online. Due to its rarity, methcathinone findings in biological specimens usually arouse suspicions.

Low levels have occasionally been found of methcathinone in blood or urine during forensic drug screening by gas chromatography mass spectrometry (GCMS). There is usually an overload of ephedrine or pseudoephedrine present in these cases, and many do not confirm when methcathinone is tested directly. These observations suggest that methcathinone can form as an artifact during GCMS analysis if high concentrations of ephedrine or pseudoephedrine are present.

The Navy Drug Screening Laboratory reported a similar issue in 1993. Proficiency urines spiked with pseudoephedrine were reported as positive for methamphetamine. Further investigation revealed that GCMS injection above 220°C promoted the loss of a hydroxyl from derivatized pseudoephedrine to form methamphetamine. The addition of a preparatory acetylation or oxidation step was suggested to remove ephedrine and pseudoephedrine in order to avoid false positive results for methamphetamine. While the oxidation of pseudoephedrine does eliminate the possibility of methamphetamine formation, it can also create methcathinone by converting the hydroxyl to a carbonyl group.

Pharmacokinetic studies of methcathinone have established that it is primarily reduced to form ephedrine. This is one explanation for why ephedrine (or its stereoisomer pseudoephedrine) is almost always detected when methcathinone is present in biological specimens. Unfortunately, this creates a chicken-and-egg situation, making it difficult to determine if methcathinone was intentionally ingested or if it might have formed in vitro due to oxidation of ingested ephedrine/pseudoephedrine. A previous report established that ingestion of 60 mg pseudoephedrine did not produce detectable levels of methcathinone in urine. Higher concentrations were not tested.

A series of experiments were conducted to determine the role of analytical conditions in methcathinone formation. Spiked blood samples were analyzed by GCMS and liquid chromatography tandem mass spectrometry (LCMSMS). Neat standards of pseudoephedrine and ephedrine were also tested to exclude the possibility that methcathinone was present as a contaminant in the ephedrine and pseudoephedrine standard materials.

Methcathinone was detected by GCMS from 20 mcg/mL ephedrine and 40 mcg/mL pseudoephedrine in spiked blood samples. Trace amounts were present at ten-fold lower concentrations. Surprisingly, methcathinone was also detected by LCMSMS in blood samples spiked with 40 mcg/mL pseudoephedrine. The methcathinone did not come from the standard material because neat injections of 50 and 100 mcg/mL ephedrine and pseudoephedrine were negative for methcathinone (LOD 1 ng/mL). Instead, methcathinone appears to form as an artifact due to interactions with the biological matrix.

It is important to consider the possibility of in vitro oxidation when methcathinone is detected in the presence of pseudoephedrine/ephedrine. This combination looks very similar to actual methcathinone ingestion since ephedrine is the major metabolite of methcathinone. However, it is possible for methcathinone to form during analysis of specimens that contain high concentrations of pseudoephedrine/ephedrine. Methcathinone formation can be minimized by using analytical procedures that avoid excessive heat (LCMSMS).

Methcathinone, Artifact, Stability

K23 Thiosulfate Antemortem and Postmortem Blood Concentrations Following Suspected Hydrogen Sulfide Exposures - An Evaluation of Ten Positive Cases

Lee M. Blum, PhD, Laura M. Labay, PhD, and Marianne T. Flanagan, NMS Labs, Inc., 3701 Welsh Road, Willow Grove, PA 19090*

After attending this presentation, attendees will learn about hydrogen sulfide as a toxic agent, the recommended specimen types that should be collected for toxicological analysis following a possible exposure and the application of thiosulfate as a biomarker of exposure.

This presentation will impact the forensic community by providing additional information that may be of benefit when evaluating cases of hydrogen sulfide gas exposure.

Situations involving hydrogen sulfide gas exposures are frequently encountered in the practice of forensic toxicology and the interpretation of analytical findings is directly dependent upon selecting the appropriate marker of exposure. Hydrogen sulfide is a colorless, flammable gas that is highly toxic. It is a natural gas that can be produced by decaying organic matter or as a by-product of various industrial processes including petroleum refining and mining. Hydrogen sulfide is insidious in that even though the gas has a distinctive rotten-egg odor that may be detectable at concentrations as low as 0.5 ppb, olfactory fatigue, depending upon concentration and length of exposure, may also occur. At room temperature hydrogen sulfide is a gas and, since it is heavier than oxygen, it tends to accumulate in poorly

ventilated low-lying areas. Inhalation is the major route of exposure, and its mechanism of action is such that it causes disruption of the mitochondrial electron transport system. In the body hydrogen sulfide is rapidly metabolized to its major oxidation product thiosulfate and it is mostly for this reason that this metabolite has often been used as a biomarker in the evaluation of non-fatal and fatal hydrogen sulfide exposure cases. Also, even though sulfide may also be used as an indicator of exposure, the detection of sulfide is difficult especially in non-fatal cases since it undergoes rapid metabolism in the body. Another potential complexity involving the interpretation of a postmortem sulfide level is that sulfide may be formed during the decomposition process.

Over the course of a 5-year period several blood specimens collected for investigative purposes where hydrogen sulfide exposure was suspected, were submitted to NMS Labs for thiosulfate analysis. The analytical technique utilized for this work is ion chromatography (IC) and the test, as it specifically relates to the analysis of a blood specimen, is briefly described as follows: specimens first undergo a two-fold dilution using deionized water followed by vortexing and centrifugation. Specimens are passed through an ultrafiltration device and the filtrates transferred to autosampler vials that are crimped with Teflon-lined caps. Identification is based upon retention time (RT) and peak shape (e.g., area to height ratio) as compared to that of calibrators and quality control samples. All analytical work is performed using the technique of standard addition and the final analytical result is reported based upon this calculation. The lower limit of quantification (LLOQ) is 2 mcg/mL.

A review of our testing results revealed ten positive cases where thiosulfate was detected in antemortem and/or postmortem blood specimens. Out of these ten cases, postmortem blood was tested in eight cases while antemortem blood was tested in three cases. It should be noted that one case had both antemortem and postmortem blood submitted for testing. The thiosulfate postmortem blood concentrations in the eight death cases ranged from 2.3 to 100 mcg/mL (average 25 ± 37 mcg/mL, median 7.0 mcg/mL). The three antemortem specimens were positive at a concentration slightly less than the reporting limit of the assay (approximately 1.6 mcg/mL), 5.8 mcg/mL and 17 mcg/mL. The specimen reported at 5.8 mcg/mL was collected from an individual believed to have been chronically exposed to hydrogen sulfide gas in the workplace. The 17 mcg/mL specimen involved the death of an individual where the sample was collected antemortem. As a point of reference, whole blood thiosulfate concentrations in healthy persons are normally less than 0.3 mcg/mL.

Although the majority of the above cases involved suspected occupational exposures to hydrogen sulfide, there was one distinctive case where two commonly used household cleaning products were purposefully mixed together so that hydrogen sulfide gas was generated in an apparent suicide. It was this case where the highest concentration (100 mcg/mL) of thiosulfate was detected.

Even though thiosulfate is not a unique marker of hydrogen sulfide exposure its detection and measurement may aid in the verification of a hydrogen sulfide exposure especially when case history supports the analytical finding.

Thiosulfate, Hydrogen Sulfide, Gas Exposures

K24 The Application of CE- and CEC-TOF/MS to the Analysis of Non-Traditional Drugs Used to Facilitate Sexual Assaults

Jennifer Greaux, BS, and Bruce R. McCord, PhD, Florida International University, Department of Chemistry, 11200 Southwest 8th Street, Miami, FL 33199*

After attending this presentation, attendees will become aware of a wide range of “non-traditional” drugs which have the potential to be used to facilitate sexual assaults as well as newer techniques for analyzing these drugs.

This presentation will impact the forensic science community by detailing the analysis of various drug mixtures to show that CE and CEC are both efficient and reliable techniques for the detection of drugs in sexual assault samples. It is hopeful that the techniques can then be used to aid authorities in prosecuting criminals accused of sexual assault in a quick but efficient manner.

The overall purpose of this project is to develop and optimize methods for the analysis of drugs which may be found in blood and urine specimens from sexual assault cases. Capillary electrophoresis coupled to electrospray ionization time-of-flight mass spectrometry (ESI-TOF-MS) permits the rapid separation and identification of these drugs. In addition, CE provides high efficiency due to its plug-like flow, which is valuable when separating mixtures. The use of capillary electrochromatography (CEC) coupled to time of flight mass spectrometry was also investigated. Because CEC is a hybrid technique of CE and HPLC, it offers both high efficiency and stationary phase selectivity. This is important when separating drugs with similar physical and chemical properties.

The first part of this project involved an optimization of injection parameters for the CE system. The drugs studied belonged to the following classes: anticholinergic (scopolamine), anticonvulsant (valproic acid), antidepressants (citalopram, doxepin, fluoextine, imipramine, paroxetine, sertraline, desipramine, nortriptyline), antihistamines (diphenhydramine, doxylamine, brompheniramine), antihypertensive (clonidine), cough suppressants (dextromethorphan), and muscle relaxants (carisoprodol, cyclobenzaprine). Mixtures of different DFSA candidate drugs and their metabolites in their salt form were prepared via a simple three step process: addition of 1% HCl in methanol, evaporation to dryness, and reconstitution of sample in water (Hudson). This provided higher sensitivities when compared to previous methods where mixtures were prepared in buffer and deionized water. In addition, a water plug was added prior to sample injection to help preconcentrate the sample via an in-line stacking process. Buffer systems examined for these analyses included phosphate and more volatile buffers, such ammonium phosphate, ammonium bicarbonate and ammonium acetate. Controlled studies were performed to determine their effect on MS signal intensity. The pH and concentrations of the buffers as well as the run voltage were adjusted to optimize CE separations. The mixtures were then separated by CE-MS on a fused silica capillary (50 µm internal diameter, 84.5 cm total length). Run times provided by the system were under 15 minutes, with UV detection possible in the first 5 minutes of the analysis. There appeared to be some overlap between peaks; however, the drugs were still able to be identified by the mass spectrometer based on their mass-to-charge ratio.

In the second part of this project, monolithic capillary electrochromatographic (CEC) stationary phases were developed to improve the selectivity and efficiency of the analysis of this group of compounds. These stationary phases were prepared in-situ via polymerization of various monomers in the presence of porogenic materials, creating stationary phases with high surface areas and good porosity. These properties also permit drug preconcentration prior to analysis. Stationary phases were tailored to provide specificity by changing the type of retentive monomers and porogenic solvent used during the polymerization process.

The analysis of various drug mixtures will be detailed to show that CE and CEC are both efficient and reliable techniques for the detection of drugs in sexual assault samples. It is hopeful that the techniques can then be used to aid authorities in prosecuting criminals accused of sexual assault in a quick but efficient manner.

Capillary Electrophoresis, Mass Spectrometry, DFSA

K25 Inline Derivatization and Detection of Primary and Secondary Amine Containing Drugs Via CE-LIF

Britt E. Turnquest, BSc, and Bruce R. McCord, PhD, Florida International University, Department of Chemistry & Biochemistry, Florida International University, 11200 Southwest 8th Street, Miami, FL 33199*

After attending this presentation, attendees will be able to understand the mechanism by which drugs containing primary and secondary amine groups can be derivatized on-capillary using 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) for the purpose of detection using capillary electrophoresis with laser-induced fluorescence.

This presentation will impact the forensic science community by providing a method that can be an excellent screening procedure for trace levels of amphetamines and other drugs in body fluids.

In forensics capillary electrophoresis has become an increasingly common analytical method due to its ability to be coupled to a variety of detection systems. One such method of detection is through laser-induced fluorescence which can provide high sensitivity and specificity in spite of the short path length used. This is particularly useful for the detection of compounds that undergo extensive first-pass metabolism and thus are present at trace levels in biological matrices. Unfortunately, compounds which fluoresce naturally are few and in order for them to be detected by fluorescence derivatization is necessary. This presentation will permit attendees to understand the mechanism by which drugs containing primary and secondary amine groups can be derivatized on-capillary using 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) for the purpose of detection using CE-LIF. NBD-F is a non-fluorescent compound which reacts to primary and secondary amines through a nucleophilic reaction whereby the fluorine attached at the benzene ring is lost and the amine group of the analyte loses a hydrogen atom and subsequently binds at that site. The resulting derivative is strongly fluorescent and has an emission wavelength around 530 nm.

Many drugs commonly feature amine groups within their structures which can be primary, secondary or tertiary in nature. The authors have focused on four specific compounds (3, 4-methylenedioxyamphetamine, 3, 4-methylenedioxymethamphetamine, norephedrine and ephedrine) that represent primary and secondary amines as well as two distinct chemical structures. It is to be noted that these compounds are either pre-cursors to or commonly encountered illicit “designer” drugs. To determine the capability of the selected fluorescent tag derivatize the analytes of interest in this study and optimize the reaction kinetics, an offline procedure was used based on work previously done by Lurie. These kinetic studies looked at derivatization temperature and time, drug concentration, molar ratio of tag to analyte, tag concentration and buffer pH.

Drug standards for each drug were obtained from Cerilliant and diluted to concentrations of 1 µg/mL. 75 µL aliquots of the drug, 20 mM NBD-F freshly prepared in ethanol and 50 mM sodium tetraborate buffer at pH 6.5 were combined in a 1:1:1 ratio and placed in a thermocycler for 10 minutes at 60°C. Samples were then hydrodynamically injected at 0.3 psi for 5 seconds into a fused silica capillary of 50 µm inner diameter, 40 cm length, 30 cm effective length. Separation took place using 50 mM $Na_2B_4O_7$ with 10 mM sodium dodecyl sulfate buffer at pH 8.5 at an applied potential of -15kV for 5 minutes. All steps in the CE method used reverse flow and polarity in order to shorten the effective length of the capillary to 10 cm. Fluorescence is then induced using an argon laser at 488 nm and separation is done using the Beckman Coulter P/ACE MDQ system. This procedure produced detection limits in the pg/µL range for each of the mentioned analytes.

Given the lack of fluorescence of NBD-F prior to derivatization, the detected fluorescence intensity can be used to quantify the amount of the analyte present. Differences in mobility between compounds and subsequently elution time can be used to determine the identity of the analyte(s) in question. The method proposed by the authors would incorporate an electrokinetic mixing step to facilitate a fast reaction between the analyte(s) and the tag after a hydrodynamic sandwich injection. As this method is geared towards urine samples where analytes of interest are in trace quantities the drug samples would be isolated via a liquid-liquid extraction procedure and reconstituted in run buffer prior to injection into the instrument.

On-Capillary derivatization, Amines, Drugs

K26 Sample Collection Tips for Automated and Comprehensive Drug Analysis in Biological Specimens Using LC/MS/MS

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After attending this presentation, attendees will learn how to automate sample preparation for biological specimens using minimal manual labor. Most importantly, the method is comprehensive for basic, acidic, and neutral drugs.

This presentation will impact the forensic science community by demonstrating how this automated method permits the possibility of forensic labs to improve chain-of-custody of samples, and increase confidence of results due to automation and tandem mass spectrometry.

The SC-Tips for biological fluids are pipette tips that contain a screen, an absorbent material, and a cap. The screen is used to contain the absorbent material and to filter subsequent solutions during automated extractions. The absorbent material serves two major purposes, to absorb and to remove sample matrix components including salts and proteins. The cap comprises a thin membrane to contain the biological samples, has grooves for robotic transportation, and an entry for a syringe needle. A multi-purpose sampler (MPS) is used to process extractions directly from the SC-Tips into LC vials and perform automated injections into the LC/MS/MS instrumentation.

The automated liquid extraction process takes approximately 1 minute to perform. By using an automated dry down station, the total extraction time is less than 6 minutes, which is less than the chromatographic analysis time. Therefore, the extraction of one sample is being performed during the chromatographic analysis of the previous sample, so high throughput is achieved one sample at a time.

For analysis of urine specimens, the samples are first pre-treated with enzymatic hydrolysis. Afterwards, 0.5 mL of acetonitrile (with spiked internal standards) and 0.26 mL of the hydrolyzed urine solution (0.2 mL equivalent of urine) are transferred to the top of the SC-Tip, and then the cap is added to close the tip. The tip is then placed on the sample tray of the MPS for robotic liquid handling. There is no other manual labor for this sample preparation. Oral fluid is prepared similarly to urine except hydrolysis pre-treatment is not required.

For blood specimens, 0.2 mL is transferred onto the absorbent material of the SC-Tip, then 0.5 mL of acetonitrile with spiked internal standard is dispensed into the SC-Tip either manually (before adding the cap) or robotically (after adding the cap). The use of the absorbent material and acetonitrile combine to precipitate proteins, remove salts, and provide a clean extract with reduced ion suppression.

High recoveries are shown for the analysis of opiates and opioids, benzodiazepines, barbiturates, stimulants (amphetamines and cocaine), analgesics (propoxyphene, tramadol), hallucinogens (PCP, THC), and muscle relaxants (carisoprodol, meprobamate). The comprehensive nature of this extraction is exemplified by simultaneous extraction of over 40 pain management drugs in a single specimen.

Duplicate or triplicate analyses of specimens can be readily performed by using 2 to 3 SC-Tips per sample without significantly increasing labor, providing better quality and confidence of results. This

may be relevant to forensic toxicological specimens, where the analytical quantitative results include the error associated with the analysis. Furthermore, the use of bar code labels on the SC-Tips ensures sample integrity and minimizes possible mishandling errors and chain-of-custody issues.

Recoveries and %RSDs for over 60 drugs are shown, with most recoveries and %RSDs being greater than 70% and less than 10%, respectively.

Sample Preparation, LC/MS/MS, Automation

K27 Ricin-Binding Proteins in Buccal Cells and Salivary Fluid

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After attending this presentation, attendees will learn how specific binding proteins for the toxin ricin can be identified in human buccal cells and cell free saliva.

This presentation will impact the forensic science community by demonstrating how the characterization of ricin binding proteins in salivary fluid and buccal cells proteins may facilitate discovery of methods for diagnosis of ricin poisoning and clarify additional details of mechanisms involved in ricin toxicity.

The plant protein ricin is one of the most poisonous known substances, is subject to biological and chemical weapons bans and is of concern as a tool of terrorists. There is no cure for ricin poisoning and diagnostic difficulty in distinguishing its effects from other harmful agents. Routes of exposure include ingestion, inhalation and injection. There are gaps in the knowledge of specific molecular identities of cell surface ricin-binding proteins. This research describes binding of ricin and the related lectin RCA-I to proteins in buccal cells and salivary fluid which are biological material that could be exposed to ricin during poisoning.

This study investigated if binding of ricin could be detected to buccal cell surfaces, salivary and buccal proteins and identification of molecular masses of ricin ligands. Whole saliva was collected by expectoration and salivary fluid and buccal cell fractions isolated by centrifugation. Ricin and RCA-I-binding proteins were detected by lectin blotting after SDS gel electrophoresis of saliva and buccal cell proteins and also measured by Enzyme-linked microtiter plate binding assays. Fluorescence microscopy with biotinylated ricin and RCA-I was used to visualize localization of ricin and RCA-I binding to buccal cell surfaces.

After electrophoresis, lectin blots identified a 170kDa buccal cell protein band in reduced samples that bound to ricin, binding was absent or decreased in non-reduced samples. Major ricin-binding proteins in salivary fluid included 170-150kDa, 75kDa, 50kDa, 40kDa and 25kDA molecules. Neuraminidase from Clostridium perfringens increased the binding of ricin to blots of salivary fluid proteins but had less effect on the binding of RCA-I. Treatment with neuraminidase from *Vibrio cholerae* did not affect the binding of ricin and RCA-I to buccal cell proteins in lectin blots. In fluorescence microscopy and microtiter plate binding assays, ricin bound only weakly to buccal cells in contrast to strong staining and binding seen with RCA-I.

Specific ricin and RCA-I –binding salivary and buccal cell proteins can be detected by lectin blotting after electrophoresis including a common 170kDa protein. There are differences in the reactivity patterns of the related molecules RCA-I and ricin with buccal cells and saliva, even though in the literature both are reported to bind to galactose-terminated oligosaccharide structures on proteins and glycolipids. Binding to buccal cells and salivary proteins could be relevant to the bioavailability of ricin or dose reaching other tissues in the event of poisoning by the oral route.

Ricin, Toxin, Saliva

K28 Workplace Toxicity In the Archives of Ottoman Empire

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After attending this presentation, attendees will understand the residual effects of multiple applications of chemical products, including heavy metals, pesticides, and rodenticides, over a five century period and its affects on archive employees.

This presentation will impact the forensic science community by demonstrating workplace toxicity due to multiple applications of pesticides and rodenticides.

The employees of the Ottoman Archives are exposed to different molds and chemical products such as heavy metals, pesticides, and rodenticides. The goal of this study is to investigate the inorganic elemental composition of archived papers to predict whether if there is any toxicity or not in the 100 to 500 years old Ottoman Archives as a work place.

Material and Method: Five ml of 70 % HNO3 and 1 ml of concentrated HCl were added to the 0.1 g aliquots of the collected paper samples from randomly chosen fifteen departments of the archive and nails from the randomly selected ten employees and digested in microwave oven under 170º C/400 watt/15 minutes. Thirty-five elements of the collected pieces of papers of each of fifteen archive rooms and a blank plain paper have been analyzed and compared by using ICP MS technique.

The ICP-MS conditions were as follows: Rf power:1200 w; Nebuliser gas flow: 0.87 ml/min; Auxiliary gas flow: 0.75 ml/min; cooling gas flow: 13.8 ml/min. sample uptake: 60 s; Dwell time: 10 ms.

Results: Average values in ppm of fifteen archive rooms for related elements have been found as follows Li : 0,1 Be: 0,174 B: 29,1 Na: 1659,0 Mg: 3104,7 Al: 9538,2 P: 652,5 S: 0,0 K: 3047,7 Ca: 10573,4 V: 3,8 Cr: 97,7 Mn:68,0 Fe: 13857,1 Co: 7,8 Ni: 24,3 Cu:82,6

Zn:312,9 As: 24,6 Se: 0,3 Sr: 51,3 Zr: 1,5 Mo: 1,4 Cd: 0,3 Sn: 14,2 Sb: 1,4 Ba: 384,6 W: 0,1 Pt: 0,0 Hg: 4,9 Tl: 0,1 Pb: 282,8 Bi: 5,1 Th: 0,676 U: 0,344 respectively.

Conclusion: This study showed that, toxic metals such as As, Cu, and Pb varied between 100 and 1,000 folds of the nowadays produced plain (blank) paper. Employees that working for long times in restoration or examination of the archived papers inside the archive rooms subjected to chronic workplace heavy metal toxicity. Furthermore from the analysis of their nail samples, employees are under the risk of heavy metal toxicity. On the other hand, 160 employees of the archives have been sent to the department of thoracic medicine where breathing functions were administered. When compared, the values in the patients files of the hospital; Forced Expiratory Volume in One Second (FEV1) breathing function although statistically not significant, decline in ten years was greater in the achieve employees, in spite of smoking was more common in the control group while other functions such as FVC, FEV1/FVC, MEF 25-75%, DLCO/VA still in normal values.

Workplace, Toxicity, Archives

K29 Preliminary Drug Screening on Postmortem Urine: An Impractical Practice

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After attending this presentation attendees will: (1) become aware of the practice of "urine-first" screening of decedents for toxicological study; (2) learn the drugs commonly detected by urine and blood drug screening; (3) be familiar with the overall sensitivity and specificity of urine drug screening on postmortem specimens; and, (4) observe a cost analysis of different protocols for postmortem drug screening; and 5. be able to form an opinion about "urine-first" drug screening based on cost and effectiveness.

This presentation will impact the forensic science community by showing how initial drug screening of urine specimens followed by reflex screening of positive urine specimens as a protocol for assessing toxicological factors in the autopsy is financially beneficial, but is too inaccurate to be of adequate quality for forensic use.

Background: In serving rural communities with limited budgets, the request often comes from the county medical examiners that screening for drugs on decedents be performed initially on urine, and if positive, reflex testing be performed on blood for confirmation. The rationale for the request is the rapid turnaround of the urine drug screen and the significant difference in cost. These requests are generally honored, since in rural counties where these data were obtained, medical examiners are very conscious of cost containment for county services. Therefore, most drug screens were performed on urine, and negative results were not further evaluated. Trying to find literature to support this "urine-first" screening practice was not successful. Therefore, we elected to perform a study of known urine and blood drug screens with discrepant results. By so doing, we hope to bring reliable data to the discussion of whether the "urine first" policy is scientifically or financially prudent.

Materials and Methods: Results of 501 autopsies were reviewed from the years 1997-2009. All cases with discrepant urine and drug blood screens were collected and analyzed. The urine drug screens were a seven-item panel that screens for cannabinoids and their metabolites, benzodiazepines, amphetamines, opiates, cocaine and its metabolite, tricyclics, and barbiturates. Blood specimens from decedents with positive urine drug screens were sent to a reference laboratory for confirmation.

Results: In all, 11 decedents had both urine and blood drug screens performed, approximately 2% of the group studied. The decedents' demographics showed 7 men and 4 women, mean age for both 39 years. Men had more positive drugs detected on urine screen than did women (20 versus 6), although this difference was not significant. There were 9 true positive tests, 17 false positives, 45 true negatives, and 5 false negatives. Thus, the sensitivity was 64% and the specificity was 73%.

Discussion: Screening urine for drugs on postmortem specimens does not appear to be an accurate way to determine which drugs were present at the time of death. It is inexpensive, however, given an example of 100 autopsies in a given period, the costs incurred for the "urine-first" protocol would be: ($36 initial urine work-up cost + $180 follow-up blood work-up cost)(36 false positives) = $7,776 spent on further working-up specimens that were positive in the absence of drugs; ($180 blood work-up cost)(27 false negatives) = $4,860 "saved" on false negatives, i.e. specimens not worked-up because they were negative in the presence of drugs; and (27 false negatives + 51 all other specimens)($36 urine work-up cost) = $2,808; total cost, $7,776 – 4,860 + 2,808 = $5,724. Compared to the price of initial screening by blood work- up for all cases ($180 blood work-up cost)(100 specimens) = $18,000, the savings are substantial, $12,276. However, the reliability of the results must be considered as well. In a screening protocol for which nearly half of the results are not accurate because of poor sensitivity and low specificity, one must make a judgment as to whether the money saved is worth the information lost, or scrambled by misleading results. Considering how many critical decisions about manner and cause of death are based on the presence or absence of drugs in a decedent, it would seem inappropriate to choose such a protocol as a routine practice.

Urine, Postmortem, Drug Screen

K30 Fatal Caffeine Intoxication: A Review of Seven Cases From 1999-2009

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After attending this presentation, attendees will understand the symptoms and postmortem toxicological assessment of caffeine intoxication. The purpose of this study was to retrospectively study all caffeine intoxication deaths over a ten year period.

This presentation will impact the forensic community by analyzing the largest series of caffeine intoxication deaths to date and highlighting the importance of testing for caffeine in postmortem samples.

Caffeine, 1,3,7-trimethylxanthine, is the most widely consumed legal stimulant given its natural occurrence in foods including coffee, tea, chocolate, yerba matte and guarana. The average content per serving is 30-60 mg per 12 ounces of a soft drink, 50 mg in 8 ounces of iced or hot black tea, 50-70 mg in 8 ounces of iced coffee and 80-120 mg in 8 ounces of hot coffee. It is estimated that average daily adult caffeine consumption is 300 mg and that moderate consumption for most adults is thought to be safe. Caffeine has been widely used in pharmaceuticals including treatment of neonatal sleep apnea, acute respiratory depression, anorectant, and most commonly for headaches and migraines. The most potent forms are available as over the counter oral caffeine tablets, each containing 100-200 mg per tablet, taken for fatigue and alertness. Rarely, serious toxicities are seen with caffeine excess, at plasma levels of 15 mg/L or higher. Toxic symptoms include weakness, vomiting, fever, seizures, cerebral edema, cardiac arrhythmias (supraventricular tachycardia or ventricular fibrillation), hypokalemia, hypocalcemia, hyperglycemia, coma, and even death. Caffeine concentrations of 80 mg/ L are considered lethal. We report seven fatal cases of caffeine intoxication listed over the past 10 years.

A retrospective database search of cases with "caffeine" in the cause of death was performed 1999 to present. All available medical records and scene investigation data were reviewed. At autopsy, heart blood and peripheral blood were collected for routine toxicological screen of 12 classes of alkaline drugs. Caffeine was detected during routine comprehensive drug testing by gas chromatography-nitrogen phosphorus detection following an alkaline extraction of the biological specimens. The presence of caffeine was confirmed by full scan electron ionization gas chromatography-mass spectrometry. Caffeine was quantified using gas chromatography-mass selective detector using spiked caffeine calibrators.

A total of seven cases were identified over the ten-year period. The subject demographics were 4 women and 3 men. There were five Caucasians, one Hispanic and one African American subjects. The average age was 49 years (range 37-57). The manner of death for two cases was classified as suicide while the remaining five cases were undetermined. The average postmortem caffeine level was 117 mg/L (range 33-320 mg/L). Isolated caffeine intoxication occurred in five cases, combined caffeine and butalbital intoxication was seen in one case

and one case had combined caffeine and alcohol intoxication. Sources of caffeine included over the counter caffeine tablets and prescription medication.

This study is the largest case series reported to date of lethal caffeine intoxication. Although caffeine is generally regarded as safe for routine use, this study clearly demonstrates that lethal intoxications can occur. Both clinical and postmortem awareness must be maintained and comprehensive toxicological testing should screen for methylxanthines to detect caffeine.

Caffeine Intoxication, Methylxanthine, Toxicology

K31 Determination of Organochlorine Pesticides Residues in Human Subcutaneous Adipose Tissue

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The goal of this presentation is to demonstrate OCP residues in human adipose tissue as a result of chronic exposure in non-agricultural people.

This presentation will impact the forensic science community by describing how chronic exposures to OCP'S may cause serious damages to an individual, in means of cancer mechanisms, as also may explain the mechanism, cause of deaths. Environmental policies will soon be a forensic discussion and insurance problem.

Cukurova region is one of the most important agricultural areas of Turkey and approximately 32% of agrochemicals is consumed in this region. Negligence of performing required safety measures and lack of appropriate equipment for the preparation, and use of pesticides frequently cause accidental, acute, or ignored chronic exposure of pesticides in Turkey. Thus, in our region, biological monitoring of the pesticides, environmentally more persistent ones in particular, has a great importance. This study was aimed to monitor the chronic exposure of organochlorine pesticide (OCs) which are highly persistent in environment, and are tend to accumulate in human tissues due to their lipophilicity and resistance to metabolism.

Gas chromatography with electron capture detector (GC-ECD) was used to identify and quantify residue levels on a lipid basis of OCs. The minimum detection limits on fat basis for the studied organochlorine pesticides were as follows: 0.48 ng/g for α-BHC, β-BHC, and δ-BHC, 0.24 ng/g for HCB, *p,p'*-DDE and 0.97 ng/g for *o,p'*-DDE, *o,p'*-DDT, and *p,p'* –DDT. Recovery studies were performed on fortified blank animal fat samples at 50 and 100 ng/g concentrations. Ten samples were examined for each concentration. Depending on the pesticide, repeated analyses showed mean values from 74 to 107% of recovery. The concentrated sulfuric acid used in the clean- up step of adipose tissue extracts in order to degrade the phthalate esters that interfere in the gas chromatographic identification of organochlorine pesticides.

The average results (±S.D) for females and males were as follows; For females: HCB 5.47 ± 6.21, α-BHC 11.27±9.89, β-BHC 3.99±5.36, Σ-BHC 12.13±10.19, *p,p'*-DDE 106.68 ±90.31, *o,p'*-DDT 1.09±0.0, *p,p'*-DDT 8.49±10.54, Σ-DDT 113.43 ±94.99 ppb. For males: HCB 5.32 ±5.57, α-BHC 6.96±5.86, β-BHC 1.22 ±0.94, Σ-BHC 4.65 ±5.67, *o,p'*-DDT 1.41 ±0.59, *p,p'*-DDE 41.07 ±38.45, *o,p'*-DDT 2.34 ±0.15, *p,p'*-DDT 3.31 ±3.33, Σ-DDT 44.02 ±40.33 ppb.

We determined dichlorodiphenytrichloroethane, and its metabolites (DDTs), hexachlorobenzene (HCB), Benzenehexachloride (BHC) residues in human subcutaneous adipose tissues of 82 autopsy cases from the Morgue Department of Adana Branch of the Council of Forensic Medicine. Of all cases, 14 were female, and 68 were male and the average age was 40.51. The relationships between the age, gender, and body mass indexes of cases, and the accumulation of OCs residues were also investigated. Detectable concentrations of *p,p'*-DDE were found in 100% of adipose tissue samples. Among the remaining *p,p'*-DDT (84.1%), HCB (62.2%) were followed by α-BHC, β-BHC, *o,p'*-DDT, *o,p'*-DDE.

Concentrations of OCs in female adipose tissues were significantly higher than male adipose tissues ($p<0.05$). Positive correlations were found between concentrations of OCs in human adipose tissues and age of cases. The obtained results were compared to the results of studies conducted in countries where pesticide use is prohibited or allowed and with similar studies performed in our country.

The present study revealed that although the use OCs is forbidden biologic monitoring still shows residues in human tissues, in Turkey. This work is highly significant, being the first study pointing out the chronic exposure to organochlorine pesticides in our region.

Organochlorine Pesticide, Subcutaneous Adipose Tissue, Gas Chromatography

K32 Concentration Distributions of the Drugs Most Frequently Identified in Postmortem Femoral Blood Representing All Causes of Death

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The goal of this presentation is to provide quantitative information about the types of drugs most commonly identified in postmortem femoral blood samples representing all causes of death. Each drug was characterized by its mean, mean and upper 90, 95, and 97.5 percentile concentrations.

This presentation will impact the forensic science community by comparing the types of drugs used and abused in Sweden with other countries.

The compilation of drugs will prove useful to compare with future cases from the same population of death cases. This allows forensic practitioners "to flag" for an unusually high concentration of a certain drug, which might be important to consider as a contributing factor in the death.

Interpreting the concentration of drugs determined in postmortem blood in terms of toxicity and whether overdosing and drug poisoning was a likely cause of death is not always easy. The circumstances surrounding the death, the police reports, eye-witness statements, the findings at autopsy and not least the toxicology report all need to be considered. People differ widely in their response to the same dose of a drug depending on pattern of absorption, dosage form, route of administration, ethnicity, enzyme polymorphism and not least previous experience with the drug and the development of tolerance. Poly-pharmacy is widespread in today's society, which increases the risk of an adverse drug-drug or drug-alcohol interaction. The concentration of a single drug might be within an accepted therapeutic range, although toxicity is exaggerated owing to concomitant use of other psychoactive substances, or because of an idiosyncratic or allergic reaction. Some drugs share the same metabolic pathways and compete for binding sites on hepatic enzymes, whereas others have similar mechanisms of action in the brain occupying receptor sites or opening or blocking an ion-channel.

Many factors determine the types of drugs identified in post-mortem specimens, including life-style, social norms and customs, availability of pharmaceutical products, media reports and advertising as well as the prescribing practices of family physicians. The popularity of recreational drugs in society, the number of forensic autopsies performed and the comprehensiveness of the analytical toxicology performed are important considerations. Drugs available on prescription in one country might not even be registered in another, as exemplified by the hypnotic flunitrazepam, which is not approved in United States but is available on

prescription in many European nations. Scheduled substances are generally more dangerous and carry a greater risk of toxicity compared with non-scheduled or over-the-counter (OTC) medication. The combined use of alcohol and central nervous system depressants, both licit and illicit, often require emergency hospital treatment.

An in-house database (TOXBASE) was used to compile a list of the drugs most frequently identified in over 25,000 forensic autopsies representing all causes of death. The age and gender of the deceased were noted as well as the types of drugs determined in femoral venous blood samples. Ethanol (> 0.1 g/L) topped the list of psychoactive substances (N = 8,108 thus 32% of cases) at mean, median and highest concentrations of 1.43 g/L, 1.20 g/L and 8.0 g/L, respectively. Acetaminophen was in second position in 11% of cases. Amphetamine and cannabis (identified as tetrahydrocannabinol in blood) were the major illicit drugs at 13th and 15th positions, respectively. Newer antidepressants, citalopram (nr 3), sertraline (nr 14), venlafaxine (nr 16) were prominent prescription drugs as were sedative-hypnotics exemplified by diazepam (nr 4), zopiclone (nr 5) and zolpidem (nr 18). Many findings of morphine and codeine in blood were heroin-related deaths as evidenced by the presence of heroin's unique metabolite 6-acetyl morphine. Finding a high morphine/codeine concentration ratio (> 2.0) in blood gives compelling evidence for a heroin-related death.

Results of post-mortem toxicology are complicated by poly-drug use, adverse drug-drug interactions, as well as a host of pre-analytical factors. This compilation of drugs and the concentration distributions should prove useful in helping toxicologists and medical examiners in deciding if a certain drug might be implicated as likely cause of death. There is only a 1 in 40 chance of the drug concentration being above the upper 97.5 percentile of the distribution. This information along with the autopsy findings and police reports will prove useful when the cause and manner of death are determined.

Autopsy, Drugs, Toxicology

K33 Determination of Lidocaine in Postmortem Cases: Direct Implication in the Cause of Death vs. Incidental Detection

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The goals of this presentation are to provide a review of the toxicity associated with lidocaine by multiple means of administration, highlight three postmortem examinations cases since 2007 in which lidocaine played a role in the cause of death, and briefly present an LC/MS/MS analytical method for both lidocaine and its primary metabolite, MEGX.

This presentation will impact the forensic community by providing information from actual case studies to better understand and interpret the role of lidocaine in the cause of death. Additionally, comparative data between intentional ingestion versus administration of lidocaine by emergency medical services personnel will be presented to determine if there are any distinguishing factors in the measurements of lidocaine and MEGX.

Lidocaine was discovered in 1948 and today has gained widespread use as a local anesthetic and antidysrhythmic. Lidocaine poisoning results in central nervous system toxicity primarily manifested by seizures and potentially respiratory arrest. Cardiac toxicity may follow to include atrioventricular block, arrhythmias, and cardiac arrest. Toxic events have been reported via multiple routes of administration including subcutaneous, intravenous, and topical. Oral ingestion is considered to be particularly toxic due to extensive first-pass metabolism to MEGX, which is as or more toxic than lidocaine itself and may accumulate due to slower elimination.

Cases are screened for lidocaine by a standard alkaline liquid-liquid and back extraction procedure and analysis by gas-chromatography-mass spectrometry. Quantitative analysis employed a single-step liquid-liquid extraction with data collection performed by LC/MS/MS on an Applied Biosystems API2000. Separations were conducted using an isocratic mobile phase on a Phenomex Synergi Polar RP column (75 mm, 2 mm, 4 micron). The LC effluent at 0.250 ml/min was introduced to the mass spectrometer via electrospray ionization.

Of the three cases investigated by our office, the first is a suspected case of lidocaine substitution for cocaine. The decedent was a 19-year-old Hispanic male, moderately decomposed, found at his place of residence nude and in front of a laptop computer. Large amounts of white powder and marijuana were observed in the kitchen. Two samples of the powder were tested and both contained lidocaine, benzocaine, and procaine. A trace of cocaine was found in one of the powders. It is unknown if the decedent knew that the primary component was lidocaine. The cause of death was ruled as lidocaine intoxication, manner accident.

The second case involved a 30-year-old Caucasian male with a history of ulcerative colitis who had been hospitalized for six days due to oral ulcers and pain and difficulty swallowing. Treatment included lidocaine and he was discharged with medications including 2% oral viscous lidocaine and Lortab elixir. Early the next morning he was witnessed to consume shots of Gatorade mixed with GHB. He was found unresponsive later that morning. The cause of death was ruled bronchopneumonia due to multiple drug intoxication manner accident.

The final case in the series involved a 27-year-old Caucasian female who was found unresponsive lying in the driver's seat of her car parked on a residential street. A box of diphenhydramine HCl and Bactine antiseptic liquid were recovered from the scene. Cause and manner of death are pending at the time this abstract was drafted; however, there is indication of lidocaine as the primary intoxicant leading to death.

Femoral blood lidocaine and MEGX concentrations the above cases ranged from 3.6 – 39 mg/L and 1.1 – 7.3 mg/L, respectively. Additional data from vitreous samples in all cases and tissue samples from case one will be presented. Lidocaine and MEGX concentrations from cases where administration by emergency medical services personnel is documented will be presented as a basis of comparison.

Lidocaine, Postmortem Toxicology, MEGX

K34 Illicit Drugs Surveillance System and Ketamine-Related Fatalities in Taiwan, 2001-2008

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After attending this presentation, attendees will learn about the illicit drug surveillance system in Taiwan and target new trend of the emerging illicit drug ketamine.

This presentation will impact the forensic science community by building on the achievements of the government's tough on drugs initiative and measuring the emerging illicit ketamine by the illicit drug surveillance system.

Ketamine, a dissociative anesthetic agent that has acquired a unique, unpleasant emergence reaction with a cardiovascular stimulant properties since synthesized in 1961 by Calvin Stevens. Recent development of pharmacology and clinical anesthesia evolves concepts of its mechanism of action and advantage of alternative routes of administration, may arise the attention of illicit drug abusers. Epidemiological studies accompanied with illicit drugs surveillance system by using illicit-drug monitor system of illicit drug-related fatalities reveal heroin (35.2%), methamphetamine (19.2%), zolipidem

(16.9%), flunitrazepam (15.7%) and ketamine (13.8%) are top five in Taiwan in 2008, and ketamine-related fatalities are only three cases in 2001 with sequential increased from 2002 (11 cases), 2003 (10 cases), 2004 (9 cases), 2005 (11 cases), 2006 (18 cases), 2007 (16 cases) and to 36 cases in 2008. Total 114 ketamine-related fatalities with 75 male (65.8%) and 39 female (34.2%) of 14391 autopsy cases during 2001-2008 are discovered. The manners of deaths of ketamine-related fatalities of accidental, homicidal, suicidal and natural cause of deaths are 57 cases (50.0%), 27 cases (23.7%), 14 cases (12.3%) and 5 cases (4.4%), respectively. Average age of ketamine-related fatalities is 27.3 years old with peak around 15-24 years old range. Increasing the multi-drugs abuse with flunitrazepam, MDMA and methamphetamine can either reduce the unpleasant or increase the risk of ketamine-related toxicity is hypothesized. The total 114 cases with incidence of ketamine taken concomitantly, was 4.4% (5 cases) for flunitrazepam, 7.0% (8 cases) for methamphetamine, and 27.2% (31 cases) for MDMA. The ketamine concentrations (mean±Std. deviation) in blood, urine and gastric content were 2.40±4.84, 3.56±5.25 and 29.34±70.43 µg/mL. The surveillance system of forensic fatalities with illicit drug monitor system can identify the emerging trend of illicit drug. Ketamine is one of the new surveillance drugs of emerging trends since 2001 that the government will continue to monitor as part of their "anti-drug" efforts.

Ketamine, Illicit Drug, Drug Abuse

K35 You Don't Look/You Don't See: Delayed Death Due to Suboxone Ingestion Involving Analysis of Alternate Non-Biological Specimens (Clothing) – The Cleveland Experience

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After attending this presentation, attendees will have a better understanding of the utilization of alternate non-biological specimens (clothing and fabric) in addition to traditional matrices (blood, urine, bile, vitreous humor, gastric) as part of the death investigation process. Utilization of clothing or relevant material is important when biological specimens from the time of death are not available.

This presentation will impact the forensic science community by reminding attendees to think outside of the normal paradigms of toxicological analysis of only utilizing biological specimens from the body or from hospital admission. In this case, the decedent survived more than a month in a coma and antemortem specimens from the drug incident were not available for analysis. Alternate non-biological specimens (clothing) impregnated with drugs from urine and vomitus were utilized as the nexus needed in clarifying the cause of death.

In 2008, a 24-year-old white female was found unresponsive at her home by her family. That evening she entered her home and asked her sister to make her a cup of tea. The sister then retired for the evening. The decedent was last seen to be seated in a chair in front of a computer. When she came into the house that evening she told her sister that she had smoked marijuana, took SuboxoneÒ and drank 3/4 of a can of beer.

The next morning she was found unresponsive on the floor next to the chair. The previous day she was in the accompaniment of a friend outside of the home. She was conveyed to the hospital with an initial diagnosis of anoxic encephalopathy secondary to possible drug overdose. The decedent had a history of drug and ethanol abuse and depression. Heroin, Suboxoneâ, and alprazolam were suspected in the overdose. She remained in an unconscious state and died approximately one month later.

An autopsy was performed at the Cuyahoga County Coroner's Office, Cleveland, Ohio. Postmortem blood: heart and femoral, urine, vitreous humor, bile and gastric were submitted for a comprehensive toxicology analysis. The initial post-mortem toxicological analysis produced a paucity of information. The blood was positive for Oxycodone 1.85 mg/L, Acetaminophen 41.9 mg/L, Diazepam 0.16 mg/L and Nordiazepam 0.08 mg/L. Fluconazole and Oxymorphone were reported as positive. All of these drugs were administered during her hospital stay. No antemortem admission blood samples were available for reanalysis. Hospital admission urine toxicology only revealed the presence of benzodiazepines, testing for Suboxoneâ was not conducted.

The family had retained the clothing that the decedent was wearing on the morning she was found. They provided to law enforcement a pair of underwear, a hooded sweatshirt and a cushion cover on which she was last seen to be seated. Previous studies have demonstrated the ability to isolate various drugs from the fibers of textile fabrics. The decedent had vomited on her sweatshirt and had urinated when she was found unconscious.

From these materials, swatches of the stained areas were cut and subjected to a comprehensive Toxicology analysis. The initial Toxicology screens of the clothing and seat cushion cover were positive for fluoxetine and caffeine, respectively.

Further testing by liquid chromatography with tandem mass spectrometry (LC-MS/MS) on the stained fabric extracts for non-routinely covered drugs was performed at NMS Labs, Willow Grove, Pennsylvania. The underwear and sweatshirt were positive for alprazolam, naloxone, buprenorphine, and norbuprenorphine. The seat cushion swatches were "negative."

In 2002 the FDA approved Suboxoneâ to treat opiate addiction. It contains Buprenorphine and Naloxone and has both analgesic and opioid antagonist properties. Suboxoneâ may dangerously increase the effects of drug-drug interactions; this includes some antidepressants, antihistamines, benzodiazepines, sedatives, analgesics, antianxiety, and muscle relaxants. Coma and death has been associated with the concomitant intravenous misuse of buprenorphine and benzodiazepines. Selective serotonin reuptake inhibitors also inhibit buprenorphine metabolism. Cytochrome CYP 3A4 interactions with azole antifungal drugs, macrolide antibiotics, and HIV protease inhibitors and may also increase concentrations of plasma buprenorphine.

The cause of death was ruled anoxic encephalopathy due to acute intoxication by combined use of Suboxoneâ, Alprazolam, and Fluoxetine. The manner of death was ruled as accidental self-administered overdose by drugs. The case is still under investigation; police are now considering criminal charges on the friend for unauthorized distribution of Suboxoneâ.

Suboxone Fatality, Death Investigation, Alternate non-biological Specimens

K36 NMR Analysis of 3,4-methylenedioxy-N-methylamphetamine (MDMA or Ecstasy) and its Metabolites in Urine

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After attending the presentation, attendees will learn about the use of NMR spectroscopy for the detection of drugs of abuse in urine. Real case studies are also presented.

This presentation will impact the forensic science community by introducing a new technique to detect and quantify the presence of MDMA (ecstasy) in human urine.

Drug testing in urine is a common technique used today. Current methods of testing urine for drugs and their metabolites include HPLC, GC-MS, or immunoassay analysis. These methods all have their drawbacks.[1] Recently, nuclear magnetic resonance, (NMR), has emerged as a means of analyzing drugs and drug metabolites in urine. There is literature precedence describing the use of NMR spectroscopy to identify compounds in urine from intoxication.[2] There are many benefits to using NMR spectroscopy: NMR is non-destructive and samples can be analyzed as many times as desired. There is also little sample preparation required.

3,4-methylenedioxy-*N*-methylamphetamine, more commonly called MDMA or "ecstasy", is a synthetic drug similar in structure to methamphetamine.

In this project, we investigated the practicality of using NMR spectroscopy to detect and quantify the presence of 3,4-methylenedioxy-*N*-methylamphetamine (MDMA or ecstasy) in human urine.

First, a calibration curve was established with spiked samples of real urine. To determine the standard deviation, seven independent urine samples spiked with the different compounds at a concentration of 0.05mg/mL were run. Variance (S^2) and standard deviation (S) of the measurements were calculated.

As for the LOD, the very nature of NMR makes it impossible to determine as it depends on the amount of scans used for the experiment. In this study the experimental time was limited to overnight experiments, allowing a quantification in the 0.01 mg/mL concentrations range.

Following this, real urine samples from MDMA users were analyzed. The real samples were collected following an IRB approved protocol. Five different samples were collected. This presentation discusses the spectra of the urine obtained from these 5 volunteers. Figure 1 shows the spectrum of the first sample. Surimposed in gray is the spectrum of MDMA spiked urine (0.50 mg/mL). All peaks for the protons of MDMA are clearly visible.

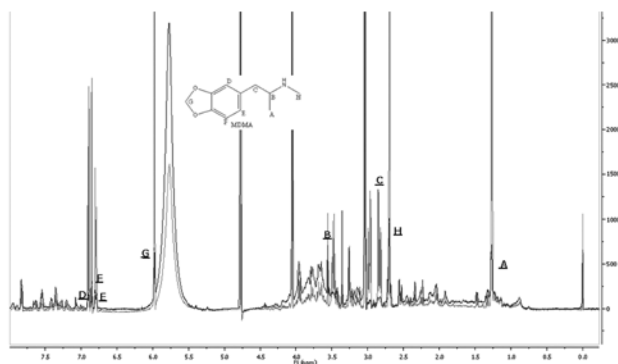

Figure 1: Sample 1, 256 scans. Superimposed in gray is the spectrum of MDMA spiked urine (0.50 mg/mL).

The results are summarized in the following table:

Sample	Time after dose	Suspected contents	Total concentration (mg/mL)	Number of scans
1	6 h	MDMA	0.082	256
2	8 h	MDMA	0.029	579
3	8 h	MDMA	0.078	128
4	7 h	MDMA	0.042	400
5	18 h	MDMA	0.045	526

These results suggest the 1H NMR spectroscopy could provide a convenient tool for the rapid detection of MDMA in human urine. This method presents the advantage of a rapid diagnosis with little of urine needed and no sample preparation. Furthermore, samples were analyzed within 20-30 minutes. The NMR method should be useful in rapidly confirming the diagnosis of poisoning.

The limitation of using NMR for the identification of MDMA is that at lower concentrations, the presence of small amounts of metabolites or other therapeutic agents can interfere. In that case, the quantification procedure can be difficult.

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NMR, Ecstasy, Urine

K37 Applications of Fire Debris Analysis to Problems in Toxicology

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After attending this presentation, attendees will understand how methods and procedures used in one of the disciplines of trace evidence, fire debris analysis, applies to problem solving in toxicology. Attendees will learn of methods and solvents to use with a case example showing the usefulness of the application.

This presentation will impact the forensic community by describing how methods and procedures in fire debris analysis could be useful to the toxicologist in certain cases. Cooperation between laboratory sections brings results unattainable by staying with existent expertise in a section. The static adsorption-elution approach should be considered by toxicologists when faced with volatile or ignitable liquid substances.

Toxicologists often use liquid-liquid or solid phase extraction to solve the majority of their case work. When faced with a poisoning case where the agent is gasoline, charcoal starter fluid, or other ignitable liquids, these typical methods fail. Ignitable liquids consist of various mixtures of alkanes, isoparaffins, aromatics, and cycloparaffins. These hydrocarbons would extract with tissue matrix consisting of fats, proteins, and cellular decomposition products soluble in organic solvents. These co-extracting matrix compounds could mask or interfere with interpreting the ignitable liquid chromatograms.

Fire debris analysts have similar problems separating the ignitable liquids from condensed pyrolysates and post-burn residues which extract similarly. By using the static adsorption-elution (SAE) method borrowed from industrial hygienists and used in fire debris analysis, a majority of the matrix materials can be separated from the compound(s) of interest. To capture the volatiles, the method uses activated charcoal strips and warming the sample to approximately 65 ^{0}C.

To elute the captured liquid, an appropriate solvent such as carbon disulfide (CS2) or n-decane can be used depending on the anticipated analyte(s). Most ignitable liquids will elute after CS2 allowing the analyst to categorize the captured liquid without the solvent interfering. Using a GC-FID or GC/MS with a DB-1 column (or equivalent) with a length of 15 meters, 0.25mm diameter and 0.25um film thickness, the GC method ranges from 40° C to 300° C for typical samples, with a 2 minute hold at the lower and upper limits. A rapid 25° per minute ramp rate allows for a short 14.4 minute analysis. Inject 1 uL of the eluted liquid. The MS is turned on at injection, turned off just before the CS2 solvent elutes, and then turned on immediately after CS2 elutes until the

end of the run. Comparison of the resulting total ion chromatogram and extracted ion chromatograms against n-alkane series and previously categorized laboratory standards of consumer ignitable liquids analyzed on the same instrument will permit classification of the ignitable liquid according to ASTM E-1618.

If the potential liquid consists primarily of low boiling compounds, a later eluting solvent such as n-decane would be suggested. Use an isothermal temperature program at low temperatures (e.g., 35° C) with the MS active from the time of injection until just before the n-decane (or other chosen solvent) elutes. Using different analytical strategies can improve detection of the volatile analytes of interest. The method can be used to determine the presence of toluene and similar compounds in huffing cases.

In one case of petroleum consumption suicide, the SAE method with CS2 was used. The male decedent a one-gallon and a one-quart paint thinner cans in his vehicle nearby. No trauma or significant levels of drugs were found. The SAE method was used to extract the lung, liver, brain, stomach contents, blood, and vitreous fluid then analyzed by GC-FID and GC/MS. The blood and vitreous did not have recognizable chromatograms. The chromatograms of the lung, liver, stomach contents, and brain eluted from the octane (C8) to the dodecane (C12) n-alkanes with at least two significant n-alkane peaks, and unresolved compounds creating a Gaussian like peak over the C8 to C12 range which was categorized in the medium petroleum distillate category. Many paint thinners are included in this category. The lung, liver, and brain each showed slight variations in their chromatograms from each other and the stomach contents. Close inspection of the stomach contents chromatograms with the liquid from each paint thinner can from the car revealed the decedent likely drank from the one-gallon container. A comparison against corresponding tissues from non-petroleum consumption deaths showed no similar compounds naturally occurring. The death was ruled a suicide by consumption of the paint thinner.

Using the SAE method can help extract volatile liquids cleanly from most toxicological matrix materials. Accomplishing identification or categorization of the captured liquid can be performed easier with a chromatogram containing fewer matrix peaks.

Ignitable Liquid, Toxicology, Analysis

K38 Development of a Method for Detecting Papain in Adulterated Urine Samples

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After attending this presentation, attendees will become familiar with a newly developed enzymatic assay for detecting papain in adulterated urine samples.

This presentation will impact the forensic community by providing a method to detect papain, a novel adulterant, in urine samples. Papain testing by this method can contribute to determining the validity of urine samples and will diminish the likelihood of individuals obtaining false negatives during drug screening due to the presence of papain in urine.

Papain is a novel urine adulterant being used to interfere with the common drug screening methods used in urine drug testing. In a study by Burrows et al., papain was found to interfere with the analysis of some drugs and was not detected in urine using current guidelines of specimen validity testing.[1] Thus, a method is needed to detect papain in urine and contribute to rendering the urine sample invalid. The current research developed an enzymatic assay for detecting papain in urine samples.

Papain is a cysteine protease that has a broad specificity, cleaving peptide bonds involving basic amino acids, leucine, and glycine. It hydrolyzes both esters and amides. A synthetic substrate, Nα-Benzoyl-DL-arginine-4-nitroanilide (BANI), was used in assay development. Papain acts on BANI to release p-nitroaniline which absorbs at 410 nm. The rate of formation of this product is easily monitored by following the change in absorbance at 410 nm. Assay conditions were established. Experiments examining the rate of product formation with varying papain concentrations and found that a linear relationship existed between the rate of product formation and concentration of papain. Papain activity as low as 0.003 units could be detected by this method. Unknown blind samples of papain were analyzed and were accurately determined as being either positive or negative for papain using this method. ***Papain itself is available from a variety of vendors and there are currently no restrictions on possession or use of papain, which makes it an easily accessible urine adulterant. However, there are also common consumer products that contain papain such as*** Adolph's® Meat Tenderizer and Beverly International® Multiple Enzyme Complex which is marketed as a digestive supplement. The assay could also detect papain in urine when these consumer products were the source of papain. The effect of storage conditions on papain activity in urine was also examined. Storage for one hour at room temperature had no significant effect on papain activity. Storage at room temperature for 2 h to 24 h led to a decrease in activity ranging from a 22% decrease at 2 h to a 48% decrease at 24 h. Storage at 4º C for 2h to 24 h led to a decrease in activity ranging from 22% at 2 h to 52% at 24 h. The results of testing for potential interference by common drugs of abuse in this papain assay will also be presented.

Reference:

[1] Burrows, DL, Nicolaides, A, Rice, PJ, Dufforc, M, Johnson, DA. Papain: A Novel Urine Adulterant. Journal of Analytical Toxicology 2005;29:275-295.

Papain, Adulterant, Urine Drug Testing

K39 Propofol Analytical Challenges and Interpretation

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After attending this presentation, attendees will have an overview of propofol concentrations reported in 71 cases between July 2008 and July 2009 along with relevant case history where available.

Propofol is a sedative-hypnotic widely used as an intravenous anesthetic agent. There is also increasing incidences of propofol abuse, especially among healthcare workers. Recently, propofol has garnered media attention and the DEA has indicated it is considering classifying propofol as a "scheduled drug", tightening restrictions on its distribution and use. Some lots of propofol were recently recalled due to contamination with suspected endotoxins. Propofol concentrations in previously reported overdose deaths were 0.22 – 5.3 µg/mL and the concentration in a homicide case was 4.3 µg/mL.

Cases submitted to NMS Labs that included a positive propofol finding were included in the analysis. Quantitative propofol analysis was performed if indicated by a gas chromatography-mass spectrometry screen performed at NMS Labs or if ordered directly by the submitting agency. Propofol quantification was conducted using capillary gas chromatography with flame ionization detection. The method has a limit of quantification of 0.05 µg/mL. Once identified, cases were reviewed and sorted according to the information initially available at the time of abstract preparation.

During a one-year period, there were 71 cases with quantifiable propofol. In two cases, propofol quantification was approximated due to sample matrix problems; these were excluded from further analysis. In the remaining 69 cases, propofol concentrations were 0.05 – 110 µg/mL (mean= 2.55 ± 13.35 µg/mL; median= 0.35 µg/mL). Forty specimens

were identified to have been collected during autopsy; the average propofol concentration in these cases was 3.29 ± 17.31 µg/mL. The elevated mean and standard deviations can be attributed to two cases with propofol concentrations of 20 and 110 µg/mL. The mean propofol concentration from central blood (0.84 ± 1.03 µg/mL) was higher than peripheral blood (0.44 ± 0.31 µg/mL) in the post-mortem cases where blood source was identified.

Of the 40 specimens collected during autopsy, case histories indicated that 12 patients were hospitalized at the time of death, five of which died while under or recovering from anesthesia. Lack of sufficient case history prohibits identification of anesthesia use in the other seven hospital deaths. The average propofol concentrations were similar for the hospitalized patients overall and those for which anesthesia use was indicated, 0.79 ± 1.03 and 0.98 ± 1.52 µg/mL, respectively. One patient who died during anesthesia induction had a propofol concentration of 3.7 µg/mL and history indicated the patient had hepatitis C. If this individual is excluded, the average propofol concentration for the remaining 4 patients was 0.30 ± 0.16 µg/mL.

Two cases were described as "suspected propofol overdose" and in a third case syringes containing propofol and fentanyl were included, though it is unclear if the patient in this case was dead. Propofol concentrations were 1.2 and 1.0 µg/mL in the suspected overdose cases and 0.20 g/mL in the case where syringes were present. In one case, propofol testing was performed based on "reasonable suspicion/cause" and a serum propofol concentration of 1.4 µg/mL was reported.

The data provided is based on information provided with case submission and thus available at the time this abstract was prepared.

Propofol, Blood Concentrations, Death

K40 Detection of Fentanyl and Lidocaine in Dried Blood Spots Using High Performance Liquid Chromatography Tandem Mass Spectrometry

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After attending this presentation, attendees will understand a unique toxicological analysis on an alternate sample matrix using HPLC/MS/MS.

The goal of this presentation is to communicate the validation and analysis of dried blood spots which were deposited onto household tissue paper. The circumstances of the donor's death were suspicious; a unique analytical opportunity was presented to the laboratory to either confirm or refute a suspect's version of the events.

This presentation will impact the forensic toxicology community in several ways. Firstly, it demonstrates that even in the absence of traditional toxicological samples such as liquid blood or urine, valuable information can be extracted from materials that more closely resemble trace evidence. Secondly, it demonstrates the sensitivity capable when state of the art instrumentation is used, suggesting that high performance liquid chromatography tandem mass spectrometry can allow for drastically reduced sample amounts. Lastly, the simplicity of the sample preparation points to streamlined extraction procedures for general drug screens.

Dried blood spots (DBS) on paper have been routinely used in DNA analysis for some time. Stain cards are routinely used by forensic laboratories for both analysis and archival of blood samples. Recently, the use of DBS samples has been also applied to clinical toxicological studies. A primary advantage of DBS analysis is that much smaller blood volumes are necessary; 15 microliters of blood can be sufficient. Another advantage of DBS analysis is the collection, transport, and storage of the stained paper material. A final benefit of the DBS analysis is the opportunity for significantly simplified sample treatment procedures due to the reduced matrix effects. The extraction employed a simple methanolic solvent extraction followed by centrifugation of a punch taken from the DBS. The success of clinical DBS analysis suggests that utility may also be found in the forensic toxicology arena.

An application of the DBS paper analysis technique was used to answer a unique analytical question posed by law enforcement during the investigation of a suspicious death. Blood stained tissue paper was recovered from the victim during the process of emergency medical treatment and law enforcement investigation. DNA analysis confirmed that the dried blood was from the expired donor. Statements from the victim's spouse seemed to be in conflict with the observed events. Establishing the veracity of the spouse's statements was dependent upon determining the precise timing of the blood deposition on the tissue paper. Emergency room medical treatment included the application of 8 drugs, including fentanyl and lidocaine. The presence or absence of these drugs in the dried blood deposited on the tissue would either confirm or refute the suspect's statements.

The analytical approach, validation, and outcome will be presented highlighting the advantages of the technique and suggesting directions for simplified drug screening methods.

Dried Blood Spots, Alternate Matrix, Tandem Mass Spectrometry

K41 Postmortem Pediatric Toxicology

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After attending this presentation, attendees will gain an appreciation for the challenges unique to toxicological findings in postmortem pediatric cases. Attendees will learn interpretive guidelines for pediatric cases involving forensic toxicology in both a general and case-specific sense.

This presentation will impact the forensic science community by further delineating the interpretive aspects of toxicological findings in the pediatric population.

In this 11th Annual Special Session within the Toxicology Section, pediatric cases involving toxicological findings are discussed. As a relative dearth exists of interpretive information involving toxicological findings in the pediatric population, this session is a forum to help elucidate and clarify such issues. The format is a short case presentation including pharmaco-toxicokinetic data and other relevant ancillary information followed by audience participation to provide interpretive clarity around the case-specific impact of the toxicological findings.

Pediatric, Toxicology, Postmortem

K42 *Melendez-Diaz* and Other 6th Amendment, Confrontational Clause Cases - Their Impacts and Perspectives

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After attending this presentation, the attendee can expect to learn about the impacts that the *Melendez-Diaz* decision from the Supreme Court has had on laboratory practice and also the perspectives of the Judiciary, prosecution and defense.

The presentations will impact the forensic science community by providing knowledge about the *Melendez-Diaz* decision and other related cases and provide important perspectives on the impacts to the laboratory, defense, and prosecution.

Throughout the years there have been many decisions that affect how scientific evidence can be entered into court proceedings with the most recognized being the *Frye* and *Daubert* decisions. The right of a criminal defendant to confront and question evidence that establishes an element of the crime of which he is accused exists in tension with the impact on laboratories that provide scientific testimony about standardized methods and results whose analysts must now personally testify about their work. The *Melendez-Diaz v. Massachusetts* decision was issued by the United States Supreme Court on June 25, 2009. While it is the latest in a line of Sixth Amendment or Confrontation Clause type cases it is the most recent decision to affect how scientific evidence can be entered into court proceedings. Specifically this case addressed the admission of evidence identifying controlled substances by affidavit. The ruling that the Massachusetts statues allowing for the admission of this evidence by affidavit was an unconstitutional violation of a defendant's right to confront and cross examine witnesses impacts every federal and state court in the United States. In doing so, the court further elucidated its position about "testimonial evidence" begun in the *Crawford* case. The implications for laboratories producing results from analytical tests and evidence generated through other disciplines will have significant impacts in some jurisdictions.

Before the end of the 2008-09 term, the US Supreme Court granted argument on another case concerning whether an witness can testify to another's work from the original analyst's report. This case, *Briscoe v. Virginia* again looks at a very similar case. Where the central question in *Melendez Diaz* is whether the Confrontation Clause requires treating crime lab reports as testimonial evidence, the central question of Briscoe is if a state allows a prosecutor to introduce a certificate of a forensic laboratory analysis, without presenting the testimony of the analyst who prepared the certificate, does the state avoid violating the Confrontation Clause of the Sixth Amendment by providing that the accused has a right to call the analyst as his own witness.

A goal of the session is to provide a forum for the discussion of the needs of forensic science evidence for each of the justice system components.

Confrontation Clause, *Melendez-Diaz*, Briscoe

K43 Brain Serotonin Transporter Reduction in Human Polydrug MDMA (Ecstasy) Users

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After attending the presentation, attendees will be alerted to the question whether use of MDMA (ecstasy) might cause damage to the brain.

This presentation will impact the forensic and general community by advising on the possible risks of MDMA use and by illustrating the need to conduct drug hair analyses to confirm use or lack of use of MDMA and other recreational drugs when investigating possible effects of MDMA on the human brain.

Background: MDMA (3,4-methylenedioxymethamphetamine, ecstasy) is an analog of methamphetamine that is widely used recreationally and is also being tested in clinical trials for the treatment of post-traumatic stress disorder. Recreational interest in MDMA is related in part to the ability of the drug to cause increased energy and sociability. Animal data indicate that chronic ecstasy exposure can cause a long term reduction in brain serotonin neurone markers, raising the public health issue of actual damage to brain serotonin neurones and associated behavioral problems in the human. However, brain neuroimaging studies in MDMA users measuring levels of a serotonin neurone marker, the serotonin transporter (SERT), have been contradictory, with most investigations not confirming by drug testing use of MDMA or other drugs.

Objective and Hypothesis: The objective was to test the hypothesis, based on animal data, that brain levels of SERT are decreased in living human MDMA users.

Methods and Subjects: SERT levels were estimated by measuring binding of 11C-DASB in a Positron Emission Tomography (PET) procedure in 50 normal subjects and 49 MDMA users. MDMA users were withdrawn from the drug for approximately 7 weeks and reported using approximately two tablets/session, two sessions/month, and 200 lifetime tablets over four years duration.

Results: All MDMA users tested positive for MDMA in hair. As expected, of the 49 MDMA users, most (40) tested positive in hair for methylenedioxyamphetamine (MDA), a metabolite of MDMA. The levels of MDA in 39 of these subjects were lower than that of MDMA, suggesting that MDA had derived from metabolism of MDMA. However, one subject demonstrated higher levels of MDA than MDMA in hair, suggesting that this subject might have ingested both MDA and MDMA.

Many MDMA users also used other stimulant drugs and there was a discrepancy between self-reported use of other stimulants vs. drug hair findings (e.g., 32/49 subjects testing positive for methamphetamine in hair vs. only 9 reporting use by self-report; 23 vs. 14 for cocaine). This discrepancy is likely explained in part by inclusion of other stimulants in tablets marketed as "ecstasy" and possibly by the expectation of the ecstasy user that he/she would more likely be included for study if other drugs were not reported as used.

Most MDMA users reported increased sociability and body temperature while on the drug (typically in a club setting) and partial tolerance developing to the behavioral effects of MDMA. Consistent with the literature, most MDMA users reported a dysphoric drug discontinuation/withdrawal syndrome (sometimes severe) occurring one or more days following last use of the drug. There was no consistent response when MDMA users were asked to report whether they were

more empathetic (caring) to others while on the drug.

Brain SERT binding was significantly decreased in the MDMA users as compared with control values, but the regional pattern was highly selective with the cerebral cortical brain regions (frontal, -27%; temporal, -27%, insular, -26%, anterior cingulate, -20%; occipital, -46%) bearing the brunt of the loss. High SERT density subcortical regions (caudate, putamen) were strikingly normal. There was marked overlap between the ranges of the control and MDMA user values. SERT binding was similar in those who tested and did not test positive for methamphetamine in hair.

Conclusions: The PET findings showing a cerebral cortical loss of SERT in MDMA users are similar to those recently obtained by a Johns Hopkins group and may help bring some consistency to this confusing literature.

Taken together, our data suggest that cerebral cortical SERT levels will be decreased, for at least two months after last use of the drug, in chronic MDMA users who use, on average, two tablets/session and two sessions/month. However, use of other drugs (methamphetamine, cocaine, cannabis) is a potentially important confound that could modify this conclusion. The observed discrepancy between recent use of drugs (other than MDMA) by self-report vs. drug hair testing also raises the possibility that other studies that do not conduct drug hair testing for stimulant drugs such as methamphetamine and cocaine may well have underestimated use of these drugs.

The special sensitivity of cerebral cortex vs. subcortical brain areas to SERT loss might be explained by differences in serotonin nerve ending characteristics or proximity to cell bodies. Finally, we emphasize that our findings cannot distinguish between actual loss of serotonin neurones and loss of SERT within intact neurones.

MDMA, Ecstasy, Serotonin Transporter

K44 Toxicological Findings in Cases of Sexual Assault in the Netherlands

Ingrid J. Bosman, PhD, Netherlands Forensic Institute, Laan van Ypenburg 6, The Hague, 2497GB, NETHERLANDS*

After attending this presentation, attendees will be provided an overview of the extent and types of drugs found in forensic cases of alleged drug facilitated sexual assault in the Netherlands.

This presentation will impact the forensic community by providing data on the most common drugs found in victims of alleged sexual assault in the Netherlands.

Reports on cases of alleged drug facilitated sexual assault have increased since the 1990s. The prevalence and types of drugs encountered during investigations of alleged sexual assault are likely to differ between countries depending on social norms and the use of drugs in society. Alcohol alone, or together with other drugs has been a common finding in many previous studies in America, Australia, the United Kingdom, and Sweden.

In this paper, the toxicological results from cases of drug-facilitated sexual assault were examined that were presented at the Netherlands Forensic Institute (NFI) between January 2004 and December 2006. The aim of this study was to identify the extent and types of drugs found in cases of alleged sexual assault. Included were those cases with an indication for sexual assault in the archives and the presence of a blood sample, a urine sample or both for analysis. Depending on the case description, information from the police, the type of biological material and the amount of sample available, analysis of alcohol, GHB and/or screening methods for drugs of abuse and prescription drugs were performed. For confirmation analysis, specific methods for the different classes of drugs were used, mostly involving GC-MS after SPE and derivatisation.

In total, 135 cases of alleged drug facilitated sexual assault were identified; 35 cases were included in 2004 and in the years 2005 and 2006 fifty cases per year were included. In 28 of the submitted cases only blood samples were available, in 50 cases only urine samples, and in 57 cases both blood and urine samples were present. Along the year, there was no clear seasonal variation in the number of reported cases of alleged sexual assault. Although the total number of reported cases is small, this is in contrast with other literature reports showing peaks during summer months or in December during the festive season. Most of the victims were female (94%) and the mean age of the victims was 25 years (range 4 – 69 years, median age 24 years).

In 27% (36 out of 135) of the cases no alcohol and/or drugs were found. The relationship between these negative toxicology results and time delay between alleged sexual assault and sampling was examined. This showed that with a time delay of less than 12 hours 11% of the cases were negative, with a time delay between 12 and 24 hours 25% and with a time delay of more than 24 hours 47% showed negative results. Therefore, some cases may represent false negative results due to the time delay.

In 108 cases, blood or urine samples were tested for both alcohol and drugs. In 47% of these cases alcohol was detected: in 22% of the cases alcohol was the only drug identified and in 25% of the cases alcohol and at least one drug were tested positive.

In 134 cases, blood or urine samples were screened for drugs. In 54% of these cases at least one drug was identified. The most common group of drugs identified was the analgesic group with paracetamol and ibuprofen being the most frequently found in respectively 27 and 16 cases. Cocaine, MDMA and THC or metabolites were the most commonly illicit drugs found. The so-called date-rape drug GHB was detected in only two cases (out of the 109 tested). Benzodiazepines were detected in 14 cases.

In conclusion, the results show that alcohol is the most commonly found drug in alleged sexual assault cases followed by analgesics, illicit drugs and benzodiazepines. Although it was not possible to distinguish between voluntary and involuntary ingestion, the presence of drugs may contribute to the victim's vulnerability. In some cases the absence of alcohol and drugs may represent false negative results due to the time delay between alleged sexual assault and sampling.

Sexual Assault, Alcohol, Drugs

K45 Validation of a High Performance Liquid Chromatography Tandem Mass Spectrometry Method for the Detection of Opioids in Hair

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After attending this presentation, attendees may evaluate the validation of an HPLC/MS/MS method for the detection of opioids in hair, and whether application of this method in their own laboratories may enhance their investigative capabilities.

This presentation will impact the forensic science community by providing another technique for the screening and confirmation of an important drug class in hair, particularly useful for the investigation of drug facilitated sexual assaults.

The detection of drugs in hair specimens poses a unique set of analytical challenges for the forensic toxicologist: limited sample amount, often vague target lists, and instrumental characteristics all impose limitations on the types of exams that may be performed upon the hair matrix. A well-validated method for the detection of opioids in hair can then serve as yet another technique for determining an individual's possible exposure to a drug, perhaps most meaningfully in drug-facilitated sexual assault (DFSA) cases.

While benzodiazepines are commonly associated with DFSA case, opioids are also used to render victims unconscious or less able to resist.

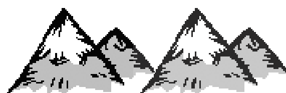
In developing a full panel of DFSA examinations, opioids should not be overlooked when the type of drug used is less clear.

The validation of an HPLC/MS/MS method for the detection of opioids in hair is presented. The method is adapted from an existing standard operating procedure previously validated by the FBI Laboratory for matrices such as blood, urine, and tissue. The adaptations necessary for the preparation of the hair matrix are discussed. The types of hair matrices included in the validation as well as various sample trial sample pretreatments are also discussed. Optimization of the HPC/MS/MS parameters is described.

The procedure allows for the screening and confirmation of morphine, codeine, hydromorphone, hydrocodone, oxymorphone, oxycodone, 6-acetylmorphine, normorphine, norcodeine, noroxycodone, dihydromorphine, and dihydrocodeine. Hair specimens are qualitatively screened and quantitated if necessary. The hair is pulverized using a freezer mill cooled by liquid nitrogen, rendering the hair to a fine powder like consistency. The specimens are mixed with buffer and internal standards, and extracted using mixed mode hydrophobic/cation exchange solid phase extraction cartridges. Target drugs are eluted using a mixed solvent system of methylene chloride, isopropanol, and ammonium hydroxide. The eluent is taken to dryness and reconstituted prior to analysis by HPLC/MS/MS.

Case studies will be presented in which the laboratory's drug screening standard operating procedures were useful in an investigation.

LC/MS/MS, Opioids, Hair



TOXICOLOGY

K1 The Analysis of PM Oral Swabs by SPE and LC–MSMS for Fentanyl as an Indicator of Administration

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After attending this presentation, attendees will learn about the usefulness and versatility of the oral swabs as a means of extracting fentanyl/norfentanyl from postmortem oral cavities. Attendees will also learn about the efficiency of solid phase extraction LC-MSMS methods in confirming these drugs in this matrix.

This presentation will impact the forensic science community by showing how fentanyl/ norfentanyl can be extracted/isolated and analyzed from swabs taken from the oral cavities in postmortem cases. This methodology will assist forensic toxicologists and pathologists when samples of blood and urine are limited.

The goal of presentation is to show how useful oral swabs taken at postmortem examination can be for the analysis of fentanyl/norfentanyl in cases when limited samples are available to analysts, forensic toxicologists, and forensic pathologists. The levels of the fentanyl (and nor fentanyl) found on the swabs are referenced against the values obtained by toxicological analysis of postmortem blood for the same cases. The data presented should add another method of analysis for facilities providing toxicological services.

In 2007-2008, oral swabs were taken from 72 post mortem cases by the pathology staff at Erie Co. Medical Examiner's Office New York where fentanyl was related to the case. In each of the cases, two swabs were employed simultaneously to extract samples from the oral cavities. The swabs were forensically sealed and submiited to Northern Tier Research (NTR). The swabs from each case were split and half of the samples were sent to Massachusettss State Police Crime Laboratory (MSPCL). These samples were used to confirm Northern Tier Research findings. Following submission to the respective laboratories, the oral swabs were extracted with 200 µL of methanol for 30 minutes in a sample tube containing fentanyl-d5/norfentanyl-d5, the swabs were washed further with a 100 µL of methanol. The swabs were removed from the sample tube before 2 mL of phosphate buffer (pH 6) were added. This solution was extracted by solid phase extraction using a mixed mode (C_8/SCX) column (200 mg, 6 mL). The SPE columns were conditioned with methanol, DI water, and ph 6 phosphate buffer (3 mL, 3 mL, 1 mL, respectively). After washing with DI water, 0.1 M acetic acid, and methanol (3 mL of each), the SPE columns were dried and eluted with: (NTR): 3 mL of ethyl acetate/acetonitrile/ammonia (78:20:2) and (MSPCL): 3 mL of dichloromethane/ isopropanol/ ammonia (78:20:2). The eluates were evaporated to dryness and reconstituted in methanol for analysis by LC-MSMS using 5µL for injection.

At NTR, tandem mass spectrometry was performed in MRM (Fentanyl: 337.2-> 188.1/105.7, Norfentanyl: 233.0->84.0/55.1) with a 50 x 2.1mm (3 µm) phenyl column. At MSPCL tandem mass spectrometry was employed in the same MRM mode using a 50 x 2.1mm (5 µm) C_{18} column. Chromatography was performed with a gradient program of acetonitrile and 0.1% aqueous formic acid at each laboratory.

Calibrators and controls were set up by extracting 0, 1, 2, 5, 10, and 7 ng of fentanyl/norfentanyl from aqueous buffer samples (2 mL) by the individual procedures. From the analysis of the calibrators and controls: r^2 value> 0.995, recoveries > 90% (NTR/ MSPCL), and a limit of detection of 0.1 ng/ mL, respectively were acheived.

Of the 72 postmortem cases where oral swabs were taken, six were confirmed to be positive for fentanyl. In two of the six cases, both fentanyl and norfentanyl levels greater than 1 ng were confirmed by LC-MSMS in both forensic laboratories (NTR/ MSPCL). These six cases were shown to have fentanyl and norfentanyl levels in blood ranging from 0.8 ng/ mL to 10.5 ng/ mL for fentanyl and 0.8 ng/ mL to 30.8 ng/ mL for norfentanyl, respectively. This data was obtained by the forensic toxicology laboratory, Erie Co. NY.

Based on data presented, analysts, forensic toxicologists and pathologists involved in post mortem cases where fentanyl and norfentanyl is suspected may wish to consider the usefulness of oral swabs in their analytical protocols. Although no direct correlation between the concentration of the drugs found in blood and those obtained from oral swabs can be drawn, this study has shown that in those cases where fentanyl was postive in oral swabs, it was confirmed in the corresponding blood samples. This relationship may be very useful in postmortem fentanyl cases.

Fentanyl, SPE, LCMSMS

K2 Rapid Quantification of THC and Carboxy–THC in Blood by LC–MS/MS

Albert A. Elian, MS, Massachusetts State Police Crime Lab, 59 Horse Pond Road, Sudbury, MA 01776*

After attending this presentation, attendees will understand a simple and improved solid phase extraction (SPE) method for analyzing THC and THC-COOH in whole blood.

This presentation will impact the forensic community by assisting forensic toxicologists/analysts in implementing a simple solid phase extraction procedure coupled with LC-MS/MS for low level quantification of THC and THC-COOH in whole blood samples.

In this procedure, after the addition of the internal standards (D3-THC and D3-THC-COOH) to 1 ml of whole blood, 2 mL of ice cold acetonitrile were added dropwise while mixing. The samples were allowed to stand for 10 minutes, after which the samples were centrifuged (10 minutes at 3000 rpm). Each supernatant was decanted into a clean tube and mixed with 5 mL of pH 7 phosphate buffer (0.1 M) prior to solid phase extraction. The mixed mode SPE columns (C_8/ SAX) were conditioned with methanol, pH 7 buffer (3, 3, mL, respectively) after which, the samples were loaded. The SPE columns were washed with 3 mL DI water, dried, and washed again with 3 mL hexanes then dried again for 5 minutes under full vacuum. Following elution of THC / THC-COOH with 2 mL of hexane: ethyl acetate (1/1), the eluents were collected and evaporated to dryness. The residue was reconstituted with 100 µl of the mobile phase solution.

Liquid chromatography was performed using C_{18} column (50x 2.1mm, 5 µm), at 0.55mL/min flow using a gradient program. The mobile phase program: (A) 0.1% aqueous formic acid) / (B) acetonitrile containing 0.1% formic acid was started at 50% (B) for 0.5 min, increasing to 90% (B) over 1.5 minute, and holding at 90% B for one minute before returning to 50% (B) and equilibrated for 2 minutes. The total chromatographic run time for each analysis was 4.5 minutes

including equilibration time. MS/MS analysis was conducted using a tandem mass spectrometer equipped with ESI in negative ion mode for THC-COOH/ D3-THC-COOH and was operated with multiple reaction monitoring (MRM) under the following conditions: curtain gas 15, collision gas medium, ion spray voltage -4500V, temperature 650 °C, ion source gas(1) 50, ion source gas (2) 50. The following transitions were monitored (quantification ions underlined): m/z 343.1 → 299.3 and 245.3 for THC-COOH, and m/z 346.1 → 302.3 and 248.3 for D3-THC-COOH. Positive ion mode was employed for THC/ D3-THC under the following conditions: curtain gas 15, collision gas medium, ion spray voltage 5000V, temperature 650 °C, ion source gas(1) 50, ion source gas (2) 50. The following transitions were monitored (quantification ions underlined): m/z 315.2 → 193.2 and 123.1 for THC, and m/z 318.2 → 196.2 and 123.1 for D3-THC.

Linearity (r^2 >0.99) was achieved from 0.25 ng/mL to 50 ng/mL, (THC/ THC-COOH) and the limits of detection were determined to be 0.1 ng/mL for THC and 0.25 ng/mL for THC-COOH, respectively. The limits of quantification were 0.25 ng/mL for THC and 0.5 ng/mL for THC-COOH, respectively. Recoveries were > 92% for THC and > 87% for THC-COOH, respectively measured at a target value of 4.0 ng/mL. Intra and inter-day precision was less than 7% and 11%, respectively for THC and less than 8% and 12%, respectively for THC-COOH. Ion suppression studies revealed that suppression of monitored ions was less than 6%.

This SPE method coupled with and fast LC-MS/MS provides a simple, sensitive, and reproducible quantitative method for the analysis of THC and its primary metabolite in whole blood. This procedure should be of great assistance to those analysts actively involved with the LC-MS/MS analysis of these drugs in biological matrices.

THC and Metabolite, Solid Phase Extraction, LC-MS/MS

K3 Simultaneous Quantification of Twenty Common Drugs of Abuse and Metabolites in Human Meconium by LCMSMS

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After attending this presentation attendees will be introduced to a liquid chromatography tandem mass spectrometry (LCMSMS) method for simultaneous quantification of common drugs of abuse in human meconium.

This presentation will impact the forensic community by offering a novel analytical method for sensitive and specific simultaneous quantification of 20 analytes in a single extraction and small meconium specimen, offering time and resource savings.

Drug abuse during pregnancy is associated with adverse obstetrical and neonatal outcomes. Detection of *in utero* drug exposure is often accomplished by meconium analysis due to ease and non-invasiveness of specimen collection and a long window of drug detection. However, the amount of meconium is often limited, prohibiting multiple assays for different drugs of abuse. Attendees will be introduced to a liquid chromatography tandem mass spectrometry (LCMSMS) method for simultaneous quantification of common drugs of abuse in human meconium.

An LCMSMS method for the simultaneous quantification of amphetamine (AMP), methamphetamine (MAMP), *p*-hydroxymethamphetamine (pOHMAMP), cocaine (COC), benzoylecgonine (BE), cocaethylene (CE), *m*-hydroxybenzoylecgonine (mOHBE), nicotine (NIC), cotinine (COT), 3'-*trans*-hydroxycotinine (OHCOT), morphine (MOR), 6-acetylmorphine (6AM), codeine (COD), hydromorphone (HYM), hydrocodone (HYC), oxycodone (OXY), methadone (MTD), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), buprenorphine (BUP), and norbuprenorphine (NBUP) in meconium in only 0.25 g of meconium was developed and validated.

Meconium specimens (0.25 g) fortified with deuterated internal standards were homogenized in acidic methanol. After centrifugation and supernatant evaporation, analytes were isolated using mixed mode solid phase extraction and analyzed by LCMSMS operating in positive multiple reaction monitoring (MRM) mode. Two analytical runs utilizing the same extract were required: a 5-µL injection, 18 minute run with gradient elution that quantified all analytes except BUP and NBUP. These two analytes were measured in a second 5 min isocratic run with a 10-µL injection volume to enhance sensitivity. The analytical method was validated over four days for limits of quantification, recovery, imprecision, extraction efficiency, matrix effects, carryover, and endogenous and exogenous interference.

Limits of quantification were 1 ng/g for COT, CE, BE, and COC, 2.5 ng/g for MAMP, EDDP, MTD, and pOHMAMP, 5 ng/g for AMP, mOHBE, NIC, OHCOT, MOR, 6-AM, HYM, HYC, OXY, and 25 ng/g for BUP and NBUP. The upper limit of quantification for all analytes was 500 ng/g, except for pOHMAMP at 250 ng/g. Correlation coefficients for each calibration curve were >0.996 with all calibrators quantifying within ±20% of target when calculated against the calibration curve. Validation parameters were tested at three concentrations spanning the linear dynamic range. Intra- and inter-day recovery ranged from 83.3 – 126.6% and 80.1 – 129.0%, respectively. Inaccuracies of up to 30% were considered acceptable due to meconium's complexity. Intra- and inter-day imprecision ranged from 0.9 – 16.9% relative standard deviation (RSD) and 3.1 – 9.8% RSD, respectively. Extraction efficiencies ranged from 46.7 – 96.0%. Matrix effects ranged from -305.7 – 40.7%, depending on the analyte, with negative values indicating ion enhancement. Matrix effects at each quality control concentration were similar for native and corresponding deuterated compounds, highlighting the importance of employing matched deuterated internal standards in LCMSMS quantification procedures, especially with complex matrices. Similar results were observed for matrix effects determination in seven different blank meconium sources fortified with low quality control concentrations; while matrix effects varied between meconium specimens, matrix enhancement or suppression of related native, and deuterated compounds were similar and quantification was within acceptable limits. Analyte stability was assessed under the following conditions: 24 h at room temperature, 72 h at 4°C, three -20°C freeze-thaw cycles, and 48 h on the 15°C autosampler. Losses of less than 34.0% were observed for each condition, except for 6AM and MOR. After room temperature, 4°C, and three freeze-thaw cycles, up to 85.8% of 6AM was lost; however, MOR concentrations under these conditions increased by up to 31.2%. In cases of suspected heroin exposure, meconium should be immediately frozen and repeated freeze thaw cycling should be avoided. No analyte carryover was observed at two times the upper limit of quantification. No interference by 57 illicit and therapeutic drugs or endogenous meconium compounds was observed. Method applicability for all analytes except 6AM, BUP, and NBUP was demonstrated by analysis of meconium from drug-exposed neonates.

The most comprehensive chromatographic method for the identification and quantification of drugs and metabolites in meconium is described. This LCMSMS method will impact the clinical and forensic community by offering a novel analytical method for sensitive and specific simultaneous quantification of 20 analytes in a single extraction and small meconium specimen, offering time and resource savings. This method will be employed in prenatal drug exposure

studies to correlate meconium concentrations to neonatal outcome measures.

LCMSMS, Drugs of Abuse, Meconium

K4 Analysis of Ethylglucuronide (EtG) and Ethylsulfate (EtS) in Urine by Liquid Chromatography Mass Spectrometry

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The goal of this presentation is to present a validated liquid chromatography tandem mass spectrometry (LC/MS/MS) method for quantitative analysis of the alcohol biomarkers, ethylglucuronide (EtG), and ethylsulfate (EtS) in urine.

This presentation will impact the forensic community by providing data obtained from a method validation study of urinary EtG, and EtS by LC/MS/MS. This study evaluated sensitivity, linearity, precision, interference, and other related parameters associated with method validation.

Measurement of ethanol in breath, blood, or urine is used in detecting recent alcohol consumption; however, ethanol is rapidly cleared from the body making it difficult to use as an indicator of alcohol use disorder. On the other hand, alcohol biomarkers, EtG and EtS, can be detected for a longer period of time making them more suitable indicators of alcohol consumption or exposure and potentially as indicators of alcohol use disorder. Samples were analyzed on a liquid chromatography system coupled to Applied Biosystems triple quadrupole mass spectrometer.

Standards spiked with concentrations of EtG and EtS ranging from 10 - 10,000 ng/mL were prepared in mobile phase and in alcohol negative urine. Urine samples (n = 14) collected from subjects following alcohol consumption were also evaluated. The LC column used for this evaluation was the Thermo Electron Corporation Hypercarb. In a previous study, the Hypercarb column showed the best chromatography for analysis of EtG and therefore was used for analysis of both markers. The LC mobile phase consisted of 5% acetonitrile with 0.1% formic acid; flow rate was set at 0.5 mL/minute. The working internal standard solution contained 550 ng/mL EtG-D5/100 ng/mL EtS-D5 in mobile phase. A 10 µL aliquot of standard or urine was mixed with 90 µL of internal standard solution. The samples were analyzed on Applied Biosystems 4000 QTrap LC/MS/MS system. The mass spectrometer was set in the ESI negative mode and analysis was performed using multiple reaction monitoring (MRM). The MS/MS ion transitions monitored were *m/z* 221 → 75 and 221 → 85 for EtG; *m/z* 124.9 → 79.9 and 124.9 → 96.9 for EtS; *m/z* 226 →75 for EtG-D5 and *m/z* 130 → 98 for EtS-D5.

The linear range was determined for this procedure by analysis on six different runs on concentrations ranging from 10 to 10,000 ng/mL EtG and EtS prepared in mobile phase and in urine. Values were considered within acceptable range if the measured amount was within ± 20% of target concentration and ±20% of ion ratio calculation. The linear range was shown to be 10 to 10,000 ng/mL for EtG and 10 to 5,000 ng/mL for EtS with a LOD and LOQ of 10 ng/mL for both analytes. The method yielded good precision for both urine and mobile phase prepared samples with RSDs of < 5% for both EtG and EtS. The present study provides a simple and rapid validated LC/MS/MS method for quantitation of the alcohol biomarkers, EtG and EtS, in urine.

Ethylglucuronide, Ethylsulfate, LC/MS/MS

K5 Validation of Opiate Detection and Quantification in Human Urine Using Liquid Chromatography and Tandem Mass Spectrometry (LC/MS/MS)

*Chelsy L. Wingate, BS**, 1517 Hawk Tree Drive, College Station, TX 77845*

After attending this presentation, attendees will have assessed the validity of a new method used in the detection and quantification of opiates in urine specimens using LC/MS/MS.

This presentation will impact the forensic community by demonstrating the development of a highly sensitive method for detection and quantification of opiates that provides rapid results, which can be utilized in both clinical and forensic toxicological settings.

The abuse of prescription pain medication has increased dramatically over recent years. Opiates, which have a high potential for addiction, are among several classes of drugs commonly used in the treatment of chronic pain. With the growing amount of opiates being used to treat pain, it is important for physicians to have the ability to monitor patient prescription use to determine if abuse has occurred. A new highly sensitive method has been developed that detects the presence of opiates in human urine specimens using liquid chromatography and tandem mass spectrometry (LC/MS/MS). This method can analyze a large quantity of samples in a short period of time due to simple sample preparation and online extraction. Alternative methods such as Gas Chromatography/Mass Spectrometry (GC/MS) require longer sample preparation time given that the sample must be extracted from the biological matrix before analysis can occur. The liquid chromatography instruments used to perform this study are multiplex systems having two to four injection ports (Thermo Scientific Aria TLX2 and TLX4) coupled with a triple quadruple mass spectrometer (TSQ Quantum Access). This multiplex system allows for analysis of a much larger number of samples than standard LC/MS/MS systems and the combination of the LC system with tandem mass spectrometry eliminates the need for derivatization, also decreasing analysis time.

In order to report toxicological results, it is crucial that the method utilized can provide reliable, reproducible results. A validation study was performed to assess the ability of this method to detect and quantify opiates accurately in urine specimens. The opiates analyzed include morphine, oxymorphone, hydromorphone, codeine, oxycodone, and hydrocodone. These six analytes are the most common opiates used in prescription pain medication. T he validation parameters evaluated in this study consist of accuracy, inter and intra-assay precision, linearity, carryover, lower and upper limit of quantification, limit of detection, and specificity.

The linearity or calibration model contained ten calibrators ranging from 50ng/ml to 50,000 ng/ml and all analytes produced an R^2 value above 0.99. The precision and accuracy was performed by analyzing five replicates at three concentrations. The precision study was performed over a three day period on two different instruments. The % CV was calculated for each day and was not to exceed 10%. All of the analytes passed this criterion except for morphine on days one and two with the lowest concentration having a CV of 13.7% and 11.94% as well as hydrocodone on day one with a CV of 11.08% for the highest concentration. Accuracy calculated based on the value determined by analysis and the true value of each analyte. All of the analytes were within 10% of the target except for oxymorphone on day one for all three concentrations and oxycodone on day two for the middle concentration with a percent accuracy of 112%. Oxymorphone is the least stable of the six opiates.

The lower limit of quantification for all six analytes was determined to be 100 ng/ml where as the limit of detection was determined to be 50

ng/ml. The upper limit of quantitation was found to be 50,000 ng/ml for all of the analytes and minute carryover was observed from extremely high concentrations of the analytes. Several different drugs such as acetaminophen, 6-acetylmorphine, methadone, buprenorphine, norbuprenorphine, morphine-3-glucuronide, oxazepam, lorazepam, alprazolam, α-hydroxyalprazolam, diazepam, and nordiazepam were analyzed for interference purposes It was determined that 6-acetylmorphine gave positive results for morphine. These results are most likely due to the acid hydrolysis step required for deconjugation. To avoid conversion from 6-acetylmorphine to morphine, it may be advantageous to use an enzyme such as beta glucuronidase for hydrolysis rather than an acid. The results from this study indicate that this LC/MS/MS method can provide reliable, reproducible results for the detection and quantification of opiates in a short period of time.

Liquid Chromatography Coupled With Tandem Mass SPE, Method Validation, Opiates

K6 Quantitative Determination of SSRI Drugs and Metabolites in Human Plasma by SPE-LC-MS/MS

Ashwini S. Sabnis, PhD, University of Utah, Center for Human Toxicology, 417 Wakara Way, Suite 2111, Salt Lake City, UT 84108; and Diana G. Wilkins, PhD, Center for Human Toxicology, Biomed Research Polymers Building, Room 490, Salt Lake City, UT 84112*

After attending this presentation, attendees will obtain valuable information about an improved, accurate, sensititve, and specific method for the quantitative analysis of the non-tricyclic class of anti-depressant drugs in human biological samples obtained from suicide decedents.

The presentation will impact the forensic community by significantly advancing our knowledge regarding the prior use of SSRI drugs in suicide decedents. This information will be a critical element in building a community-based treatment approach to preventing suicides.

Selective serotonin re-uptake inhibitors (SSRIs), a class of non-tricyclic antidepressants, are marketed as safe and effective in treating depression, anxiety disorders, and some personality disorders. Although, questions related to their safety were raised, with studies reporting a possible association with suicidal tendencies, inferences regarding the validity and strength of such an association have been divergent. The goal of this study was to develop a rapid and sensitive HPLC-MS/MS/ESI method for simultaneous determination and screening of the most commonly prescribed SSRIs in human plasma samples from suicide decedents.

A solid phase extraction (SPE) method coupled to LC-MS/MS was developed for the simultaneous analysis of 5 SSRIs, Fluoxetine (Fluox), Paroxetine (Parox), Fluvoxamine (Fluvox), Sertraline (Sert), and Citalopram (Citalo), and three of their pharmacologically active N-demethylated metabolites, Norfluoxetine (Norfluox), Norsertraline (Norsert), and N-desmethylcitalopram (Descitalo), using Waters Oasis HLB SPE cartridges. Stock solutions of the individual drugs, as well as the internal standards (I.S.), Fluox-d_6, Norfluox-d_6, Parox-d_6, Sert-d_3, Norsert-d_4, and Citalo-d_6, for calibration standards and QC were prepared in MeOH and stored at -20°C.

An LC system consisting of Agilent HP 1100 series and a Thermo/Finnigan Quest TSQ triple-stage quadrupole MS, equipped with Xcalibur (v 1.1) operating software was used for data analysis. Ionization was achieved using electrospray in the positive ionization mode. Chromatographic separation of all the compounds was achieved within 15 mins using a Waters YMC ODS-AQ C18 (150×2 mm, 3 µm) analytical column, and a mobile phase gradient consisting of 0.1% formic acid in water and MeOH at 10%, 30%, 40%, and 10% for 1, 1, 4, and 9 min, respectively. Identification and quantification were based on selected reaction monitoring.

Fluox, Parox, Fluvox, Sert, Citalo, Norfluox, Norsert, Descitalo, Fluox-d_6, Norfluox-d_6, Parox-d_6, Sert-d_3, Norsert-d_4, and Citalo-d_6 were detected by measuring transitions of m/z 310→ 148, m/z 330→ 192, m/z 319→ 200, m/z 306→ 159, m/z 325→ 262, m/z 296→ 134, m/z 292→ 159, m/z 311→ 262, m/z 316→ 154, m/z 302→ 140, m/z 336→ 198, m/z 309→ 159, m/z 296→ 160, and m/z 331→ 262, respectively. To evaluate linearity, three calibration curves over a concentration range of 1–1000 ng/mL for each of the compounds, were tested separately. A $1/X^2$ weighted quadratic curve was used for quantification. The method was fully validated, including inter- and intra-run accuracy (within 15% of target concentration) and precision (CVs <15%) for QC samples at 5, 50 and 300 ng/mL. The mean recovery for all SSRI drugs ranged from 32-74%. Stability testing showed no evidence of degradation in processed plasma samples during 3 successive freeze/thaw cycles or after storage at -20°C for at least four weeks or at 4°C after at least 24-48 hrs.

The method described herein is accurate, sensitive, highly specific, and can be used for routine therapeutic drug monitoring, toxicological screening, as well as for the study of the pharmacokinetics and metabolism of the SSRI drugs in biological specimens from normal subjects, as well as from suicide decedents.

Anti-Depressants, SSRI Drugs, LC-MS/MS

K7 Evaluation of Inter-Instrument Transferability of LC/MS/MS Methods

Tania A. Sasaki, PhD, Applied Biosystems, 850 Lincoln Centre Drive, MS 430, Foster City, CA 94404; and Adrian Taylor, MS, and Adam Latawiec, MS, MDS Analytical Technologies, 71 Four Valley Road, Concord, Ontario L4K 4V8, CANADA*

After attending this presentation, attendees will have learned the advantages and limitations of direct transfer of LC/MS/MS methods across instruments and across labs and the considerations for successful transfer of methods.

This presentation will impact the forensic community by investigating data quality when using a method from one lab and directly transferring the method to another lab. Direct transfer of methods decreases the amount of time required to implement an analytical assay in a laboratory.

The objective of this paper is to develop an LC/MS/MS method in one lab and directly transfer that method to other labs which have the same make/model of LC/MS/MS system without further optimization. Data are analyzed and variations are compared across instruments and labs.

An LC/MS/MS method was developed to detect and quantify several different drug compounds across various drug classes. After method development, the method was directly transferred to 4 different laboratories with the same model of LC/MS/MS instrument; no additional tuning or optimization of the system was performed. The inter-instrument data was analyzed and the consistency of the data evaluated. Sensitivity, ruggedness, and reproducibility were all compared.

Data analysis showed that direct transfer of an LC/MS/MS method between different instruments was possible. When sensitivity of the method was evaluated, all systems were within about 3x of each other. The biggest variable was retention time of the analytes, as it is necessary to consider several factors, such as tubing length, mobile phase consistency, and column-to-column reproducibility.

This study showed that it is feasible to develop methods and directly transfer these methods between laboratories to other instruments of the

same model. No significant variations in sensitivity or other aspects of data quality were observed. The ability to transfer methods without individual optimization of each instrument can save substantial time in method set-up and implementation.

LC/MS/MS, Method Development, Toxicology

K8 Determination and Quantitation of Noroxycodone in Human Urine Samples Using High Performance Liquid Chromatography - Electrospray Ionization-Tandem Mass Spectrometry

Christopher Doctorman, BS, University of Central Oklahoma, 100 North University, Edmond, OK 73034; and Chelsy L. Wingate, BS, 1517 Hawk Tree Drive, College Station, TX 77845*

After attending this presentation, attendees will have a greater understanding of opiate chemistry, metabolism, kinetics, and pharmacology, as well as be familiar with and implement current LC/MS/MS technology. Attendees will also gain information about an analytical method for determination of noroxycodone, a metabolite of oxycodone, and will understand the metabolic pattern for oxycodone.

This presentation will impact the forensic community by giving greater insight into human metabolism of oxycodone. This information can be utilized to perfect or improve current methods for detecting and quantifying oxycodone and its metabolites in clinical and forensic toxicological settings.

Oxycodone (4,5-epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one), is an analgesic, semi synthetic opioid derived from thebaine. Also known by its manufactured names OxyContinä, OxyNormä, Roxicodone™, and others, it comes in a variety of shapes and dosages. Oxycodone is commonly prescribed for significant pain management typically associated with cancer, and has been used clinically for this purpose in the United States for the past eighty years. It has been a "drug of abuse" for nearly 50 years.

Oxycodone is metabolized in the body by two isoenzymes Cytochrome P450 (CYP) 3A4 and CYP2D6. CYP3A4-mediated metabolism of the compound yields N-demethylated metabolites noroxycodone, noroxymorphone, and a and b noroxycodol. CYP2D6-mediated metabolism produces O-demethylation of oxycodone to oxymorphone and a and b noroxymorphol, and 6-keto-reduction to a and b oxycodol.

Human urine samples, collected as part of another study to determine the elimination rate of oxycodone, were used as test samples for the detection and quantitation of noroxycodone. A method developed for the simultaneous quantitation of several opiates, including codeine, hydrocodone, hydromorphone, oxycodone, oxymorphone, and morphine, was modified to also incorporate noroxycodone as one of the compounds using selected ion monitoring (SIM). This method was utilized on a 4-channel multiplexing HPLC system interfaced with triple quadrupole mass spectrometer. Limit of quantitation, as well as between day accuracy and precision (%deviation and %CV) of noroxycodone was established at 100 ng/mL (3.9% and 24.9%).

Urine samples were collected over a period of a week from seven individuals given one of three different concentrations of oxycodone, along with a naltrexone blockade (50 mg per day). Concentrations of noroxycodone, oxycodone, and oxymorphone resulting from the analysis of an individual dosed with 80 mg tablets of oxycodone have shown noroxycodone to be the primary metabolite (70.8%±4.7) followed by oxycodone (18.5%±5.2) and oxymorphone (10.8%±2.1). Results for samples from other individuals will be tabulated and presented. These concentration results indicate that CYP3A4 mediation is the predominant metabolic pathway of oxycodone in humans.

Noroxycodone, High Performance Liquid Chromatography, Tandem Mass Spectrometry

K9 Development and Validation of a LC/MS Method for the Determination of Guanfacine in Urine

Sara J. Kester-Florin, BS, 944 Wye Drive, Akron, OH 44303; and Carl E. Wolf, PhD, and Alphonse Poklis, PhD, Medical College of Virginia, Box 98-165, VCU/MCVH Station, Richmond, VA 23298-0165*

After attending this presentation, attendees will become familiar with a validated liquid chromatography/mass spectrometry (LC/MS) method for detecting and quantifying guanfacine in urine specimens.

Guanfacine is a drug that was initially approved for the treatment of hypertension in adults, but has been recently approved (2007) for the treatment of attention deficit/hyperactivity disorder (ADHD) in adolescents. Due to the new therapeutic use, an increase in both availability and consumption of this drug required the development of an analytical method to detect the use or abuse of guanfacine. A validation of this LC/MS method will impact the forensic community by providing the field of toxicology with a rapid, robust analytical method that requires a small sample volume, and is also sensitive enough to detect drug use at a therapeutic dose.

The validation of a LC/MS method for the detection and quantification of guanfacine in urine is presented. Guanfacine was extracted from alkaline buffered urine using a liquid-liquid extraction scheme with ethyl acetate. Two hundred microliters of samples, controls, and calibrators were prepared with the addition of 10µL of protriptyline internal standard (2mg/L). Samples were buffered to a pH of 9.5 with 200µL saturated carbonate:bicarbonate solution. Five hundred microliters of ethyl acetate was added to the samples, followed by two minutes of rotation and five minutes of centrifugation at 3000rpm. The organic layer was transferred to a clean test tube, evaporated to dryness under a gentle stream of nitrogen, and reconstituted in 200µL of mobile phase. Guanfacine and protriptyline were separated and quantified on a reverse phase S-5 micron, 2.0 x 150mm column in a high performance liquid chromatography (HPLC) separations module coupled to a mass spectrometer (MS) with electrospray ionization operated in the positive ionization mode. The mobile phase consisted of 40% 10mM ammonium formate in methanol, and was delivered isocratically at a flow of 0.3 mL/min. Sample injection volume was 10µL. The MS was operated in selected ion resonance mode (SIR) using the following m/z ions: 246, 248, and 250 for guanfacine, and 264 and 265 for protriptyline. Under these conditions the retention time for guanfacine and protriptyline were 2.1 min and 3.6 min, respectively.

The analytical measurement range for guanfacine ranged from 5ng/mL to 2000ng/mL with a 5ng/mL limit of detection (LOD) and a 20ng/mL limit of quantitation (LOQ). The method was shown to be both precise and accurate. Precision for the assay was determined at concentrations of 40ng/mL, 100ng/mL, and 500ng/mL, (n=6), the %CV was <15% for all three concentrations. Percent recovery of guanfacine was also performed using the same concentrations and was shown to be 92%, 89%, and 93% respectively. Interference with other therapeutic drugs and drugs of abuse was assessed by analyzing two controls containing known concentrations of drugs in both categories. No interferences were noted. The method was used to analyze over 100 random post diagnostic specimens from children ranging in age from 4-

to 18-years-old. Ten of the samples yielded positive results for the presence of guanfacine. Some of these samples were evaluated using a previously validated gas chromatography/mass spectrometry (GC/MS) method, and results were found to be comparable. Of the ten positive samples, seven were confirmed to be from patients who were prescribed guanfacine. No patient history was available for the remaining samples. This LC/MS method provides a rapid and reliable method for the routine determination and quantitation of guanfacine in urine specimens.

Guanfacine, LC/MS, Electrospray Ionization

K10 The Analysis of Pain Management Drugs Found in Urine Samples by LC/MS/MS

Greg A. Newland, BS, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404*

After attending this presentation, attendees will learn about a new drug screening test for the analysis of pain management drugs using LC/MS/MS technology.

This presentation will impact the forensic community by providing information that enables toxicologists to easily test for a large list of drugs that are used for management of acute and chronic pain.

A multitude of drugs have historically been used to ease the pain patients suffer with conditions ranging from cancer to arthritis. As a result many labs, both clinical and forensic, have been looking for an application to test for all of the major drugs used during the treatment of these conditions. This application covers the testing of these drugs in urine matrix by "dilute and shoot" type sample prep. The use of LC/MS/MS allows the user to do limited sample prep while still providing adequate specificity to test for more than 40 different pain management drugs in less than 8.5 min from injection to injection.

All drugs were analyzed in a single injection using a LC/MS/MS and were extracted from urine after an enzyme hydrolysis. The Limits of Quantitation differed for each drug but ranged from <5 ng/ml to 200ng/ml when extracted using a 1:10 dilution of urine samples. The linearity for each drug spiked into urine exceeded R correlation of 0.98. Each drug was analyzed using two transitions and the LOQ was based on the least sensitive of the two transitions. Ion Ratios were calculated for each ion and were <40% at the LOQ of each ion.

LC/MS/MS, Pain, Urine

K11 A Quick LC/MS/MS Method for the Analysis of Common Benzodiazepines and Opiates

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After attending this presentation, attendees will understand LC/MS/MS and its utility as an analytical technique to detect use or abuse of benzodiazepines and opiates.

This presentation will impact the forensic community by teaching about a method that is easier and has a faster turnaround time than many techniques in use today.

The objective of this paper is to develop a fast method for analysis of common opiates and benzodiazepines in urine. The method presented has a faster run time, simple sample preparation, and combines analysis of two drug classes into a single assay.

Analytes included in this method are: 6-Monoacetyl Morphine (6-MAM), Codeine, Morphine, Oxycodone, Hydrocodone, Hydromorphone, Desalkylflurazepam, Alprazolam, α-Hydroxyalprazolam, Diazepam, Nordiazepam, Lorazepam, Oxazepam, Temazepam, Triazolam, 7-Aminoclonazepam, and Clonazepam. Deuterated analogs of each analyte were used as internal standards.

Urine samples were hydrolyzed, centrifuged for 2 minutes and diluted 1:5 with LC mobile phase. LC/MS/MS analysis was performed on a Shimadzu Prominence LC stack interfaced to an Applied Biosystems hybrid triple quadrupole/linear ion trap mass spectrometer. Injection-to-injection analytical run time was 6.5 minutes. Two MRM transitions per analyte were monitored and one transition per internal standard. The Scheduled MRM™ algorithm was use for optimal method performance for this multi-analyte method.

Results showed that all analytes were successfully detected in the 6.5 minute run time utilized. The LLOQs for most analytes was around ≤5 ng/mL and all analytes had an LLOQ ≤50 ng/mL. Precision and accuracy were both within 10% except at or near the LLOQ, where both precision and accuracy were within 15%. The linear dynamic range was at least three orders of magnitude for all analytes.

An LC/MS/MS method was developed to quickly analyze common benzodiazepines and opiates in urine. The minimal sample preparation, combined with short LC/MS/MS run time drastically decreased sample turnaround time and increased throughput without compromising sensitivity or selectivity. Additionally, the ability to combine two assays into one quick LC/MS/MS run further decreased analysis times and costs.

Opiates, Benzodiazepines, LC/MS/MS

K12 A New Approach in Forensic Toxicology: Dimercaptosuccinic Acid (DMSA) Provocated Urine Potential Toxic Metal Test by Inductively Coupled Plasma - Mass Spectrometer (ICP - MS)

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After attending this presentation, attendees will understand the application of dimercaptosuccinic acid (DMSA) as a heavy metal provocateur in forensic toxicology and learn the analysis of DMSA provocated urine with Inductively Coupled Plasma Mass Spectrometer (ICP-MS) in cases of chronic heavy metal intoxication.

This presentation will impact the forensic community by serving DMSA provocated urine toxic analysis as a new approach in forensic, environmental, and workplace toxicology.

DMSA is one of the agent used as a chelator in treatment of cases of acute heavy metal intoxication. There is a notable increase between the results of ICP-MS analysis of urine samples with and without DMSA provocation in the cases of heavy metal intoxication. Urine samples with DMSA provocation had high toxic metal concentration (Hg, Pb, Ni, Ar, Sn, Sb) considering to urine samples without DMSA. Also the observation of the highest potential toxic element limits in DMSA provocated urine samples of healthy individuals was notable. After this presentation, possibility and usefulness of this new application other than authentic methods in the determination of toxic metal limits will be highlighted.

Potential toxic metal analysis can be done by investigation of provocated urine samples taken from healthy individuals. Although some other provocation agents are present, DMSA should be chosen, since it is preferred also for children. However, the administered DMSA amount should be evaluated by a clinician based on her/his health condition and physical situation such as age, height, weight, etc.

The evaluation can be done among the healthy individuals exposed toxic metals for any reason whatsoever (chronic intoxication, workplace toxicity, environmental exposure, illness, etc.). Individuals may be classified according to their living regions and appropriate precautions may be taken by determination of workplace exposure limits.

A total of 36 trace and potential toxic elements were analyzed from 10 urine samples after appropriate sampling by using elemental analysis method. ICP-MS, which has wide range usage recently as a sensitive and quick and well interpretted method, was preformed in this study.

The toxic element limits between the DMSA provocated and non provocated urine samples of children and healthy individuals was compared. On the basis of data obtained from results of this comparison, authors are in the opinion of using DMSA, which representing possible new application in the field of forensic science, environmental toxicology and workplace toxicology.

DMSA, Toxic Metal Analysis, ICP-MS

K13 The Second Seven Years of the FAA's Postmortem Forensic Toxicology Proficiency-Testing Program

Patrick S. Cardona, BA, Federal Aviation Administration, AAM-610, CAMI Building, 6500 South MacArthur Boulevard, Oklahoma City, OK 3169-6901*

After attending this presentation, attendees will have an awareness of the FAA postmortem forensic toxicology proficiency testing program its impact to the PT participants during its second seven years of existence.

This presentation will impact the forensic community by informing attendees of the positive benefits of accreditation and quality control/quality assurance for those who participate in the program.

Attendees will be acquainted with the analytical findings of survey samples of the Federal Aviation Administration's (FAA's) postmortem forensic toxicology proficiency-testing (PT) program.

For aircraft accident investigations, samples from pilot fatalities are analyzed at the FAA's Civil Aerospace Medical Institute (CAMI) for the presence of combustion gases, alcohols/volatiles, and drugs. Throughout this forensic toxicological process, a high degree of quality control/quality assurance (QC/QA) is maintained, and quality improvement is continuously pursued. Under this philosophy, CAMI started a quarterly forensic toxicology PT program in July 1991 for the analysis of postmortem specimens. In continuation of the first seven years of the CAMI PT findings reported earlier, PT findings of the next seven years (July 1998–April 2005) are summarized herein. During this period, 28 PT challenge survey samples (12 urine, 9 blood, and 7 tissue homogenate) with/without alcohols/volatiles, drugs, drug metabolites, and/or putrefactive amine(s) were submitted to an average of 31 participating laboratories, of which an average of 25 participants returned their result sheets—that is, 53–96% (mean = 82%). The number of respondents was dependent upon the complexity of the sample matrix, the number and types of analytes in the sample, and the associated analytical chemistry/toxicology. For example, ethanol/methanol/volatiles in urine were correctly quantitated by a higher number of participants than those for amphetamine/methamphetamine and cannabinoid levels in blood and tissues. Methods employed ranged from immunoassays to gas chromatography-mass spectrometry/high performance liquid chromatography. Analytes in survey samples were correctly identified and quantitated by a large number of participants, but some false positives of concern were reported as some of them were abused drugs. Some of the false positives would have been avoided by not reporting those drugs solely based upon qualitative analyses. Their presence should have been confirmed, authenticated, and, if possible, quantitated by other analytical methods, which should have been based upon different analytical principles than those used during qualitative analyses. It is anticipated that the FAA's PT program would continue to serve as a tool to effectively allow its own toxicology laboratory and other participating laboratories for professional and technical maintenance and advancement on a voluntary, interlaboratory, and self-evaluative basis. Furthermore, this PT program will continue to provide service to the forensic toxicology scientific community through this important part of the QC/QA for the laboratory accreditation to withstand professional and judicial scrutiny of analytical results.

This presentation will summarize the PT results of the participating laboratories in the field of forensic toxicology. By understanding those survey results and applying related necessary procedures, the overall performance of a laboratory should improve. Participation of laboratories in external PT programs is a realistic approach for continuous quality improvement.

Toxicology, Proficiency-Testing, Quality Improvement

K14 Quantitative Determination of Ethylene Glycol Using Capillary Gas Chromatography by Direct Specimen Injection

Trista M. Haupt, BS, Emily Lemieux, BS, and Kenneth E. Ferslew, PhD, Section of Toxicology, East Tennessee State University, PO Box 70422, Johnson City, TN 37614-0422*

The goal of this presentation is to demonstrate how this method is useful in forensic and clinical cases to determine ethylene glycol concentrations by gas chromatography.

This presentation will impact the forensic science community with a more efficient and accurate method for quantification of ethylene glycol in blood or serum.

Ethylene glycol (EG) can be accidently ingested or sometimes abused by alcoholics for intoxication when no other form of alcohol is available; in either situation, untreated poisonings from overdose can be fatal. The consequences of consuming EG range from central nervous system depression to anionic acidosis and eventually death. Pathologists commonly discover calcium oxalate crystals while reviewing EG poisonings deposited in the brain, lungs, kidneys, and heart. EG blood concentration > 20 mg/dL should receive medical treatment; > 50 mg/dL are usually associated with severe intoxication; and > 200 mg/dL have been lethal. An approximate lethal oral dose of 95% EG is 1.5 mL/kg. The goal of the present work is to develop an effective method to analytically measure EG concentrations in biological fluids. Gas chromatographic methodology was performed on a gas chromatograph (GC) equipped with an auto sampler using a 5 µL syringe for a 1 µL injection; 4mm internal diameter splitter liner (@ 235°C); a 30 meter, 0.32 mm internal diameter, 1.80 µm film thickness novel stationary phase column and a 10 meter guard column (using a time temperature program of 100-140°C at 7.0°C/min, then 140-170°C at 40 °C/min); a flame ionization detector (@ 240°C); and helium as a carrier gas (@ 1.5 mL/min). EG and 1,2 propylene glycol (internal standard) separate at retention times of 3.58 and 3.77 minutes, respectively. Time temperature programming maximizes oven temperature to ensure all biological material is eliminated following injection. Computer software was used to analyze chromatograms for peak identification and quantitation. Acetone, methanol, ethanol, and isopropanol do not interfere with the chromatography of the glycols. The method is linear over a range of 20

to 200 mg/dL. Samples above the linear range are diluted appropriately with deionized water to fit within the standard curve.

Between-day and within-day replication of three controls (37.5, 75, and 150 mg/dL) were analyzed to test the reproducibility and accuracy of the method. Results of within-day replication (n=6) of the controls were (mean concentration ± SE, coefficient of variation): 37.8 ± 0.166; 1.08%; 77.2 ± 0.307, 0.976%; and 157.2 ± 1.19, 1.86%. Likewise, results of between-day replication of controls (n=6) revealed (mean concentration ±SE, coefficient of variation): 37.6 ± 0.211, 1.37%; 76.5±0.428, 1.37%; and 153 ± 2.29, 3.67%. Determination of the limit of detection was determined by serial dilution to be 1 mg/dL. The limit of quantitation for the method yielded a significant concentration of 5 mg/dL. The usefulness of this method was confirmed by application to clinical specimens. Case in point, a 39-year-old male was admitted to the hospital after consuming EG. EG blood concentrations were determined using this method ranging from 382 to 67 mg/dL. EG was removed from the patient's circulation by hemodialysis to an undetectable concentration over a four day period and physicians were able to stop treatment. GC of biological fluids by direct injection onto a capillary column has proven to be an effective, sensitive, and accurate method for determining EG blood concentrations. Distinct advantages of direct injection, capillary GC over other methodologies is that it is rapid, does not require any special specimen preparation and only requires a minimum of 10 µL of specimen. This method is useful in forensic and clinical cases to determine EG concentrations.

Ethylene Glycol, Gas Chromatography, Capillary

K15 A Simple Liquid - Liquid Extraction of Carisoprodol and the Metabolite Meprobamate From Suspected Blood and Urine DUI Specimens for GC/MS Analysis

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After attending this presentation, attendees will understand a modified and improved method for analyzing carisoprodol and the metabolite meprobamate in blood and urine specimens. The goal of this presentation is to demonstrate a quick, clean, and effective liquid-liquid extraction method to detect the presence of carisoprodol (Soma®) and the metabolite meprobamate in blood/urine specimens at levels above, below, or at therapeutic concentrations, in turn, providing supportive analytical data for the assessment of suspected DUI cases. Literature data indicates severe driving impairment and intoxication when the combination of the two drugs exceeded 10 mg/L, a level that is still within normal therapeutic range.[1] Ultimately, this extraction method will prove a number of advantages compared to previously reviewed extraction methods.[2]

This presentation will impact the forensic community by demonstrating a clean and effective gas chromatography/mass spectroscopy (GC/MS) based and validated method for detection of carisoprodol (Soma®) and the metabolite meprobamate in suspected driving under the influence (DUI) specimens.

Carisoprodol is a commonly prescribed muscle relaxant that has not been classified as a controlled substance. The Brazoria County Crime Laboratory has observed that in suspected impaired drivers, the frequency of blood/urine specimens testing positive for carisoprodol and the metabolite meprobamate has increased over the past few years. This data reflects the obvious need for a simple, validated extraction method to confirm carisoprodol and the meprobamate in suspected impaired drivers.

To demonstrate a quick, clean, and effective liquid-liquid extraction method to detect the presence of carisoprodol and the metabolite meprobamate in blood/urine specimens at levels above, below, or at therapeutic concentrations, in turn, providing supportive analytical data for the assessment of suspected DUI cases. Literature data indicates severe driving impairment and intoxication when the combination of the two drugs exceeded 10 mg/L, a level that is still within normal therapeutic range.[1]. Ultimately, this extraction method will prove a number of advantages compared to previously reviewed extraction methods.[2]

In this method, samples were prepared by adding buffer, barbital (internal standard) and chloroform to 250 uL of specimen. Barbital is the recommended internal standard due to the fact that it does not co-elute with targeted drugs of interest and is compatible with systems other than GC/MS, such as flame ionization detection (FID). The extraction efficiency and linearity of carisoprodol and meprobamate were analyzed at levels consistent with DUI blood/urine by comparing different buffer systems and adjusting pH levels. Buffer systems and pH adjustments evaluated were 0.1 M acetate buffer pH 4.5 and 0.1 M acetate buffer pH 4.5 saturated with NaCl, 1.0 M acetate buffer pH 4.5, and 0.1 N HCl. A five point calibration curve including 4mg/L, 10mg/L, 30mg/L, 40mg/L, and 60mg/L was utilized to determine linearity. After mixing, the chloroform was pipetted into a clean test tube and evaporated to dryness under nitrogen. The residue was reconstituted with 120uL of ethyl acetate and analyzed using an Agilent Technologies 6890 GC coupled to a 5975 MSD in electron sensitive-selective ion monitoring (EI-SIM) mode for quantitative analysis. GC injection conditions were evaluated under splitless, split, and pulsed-split modes.

The evaluation of this extraction method was based on precision, cleanliness, and chromatographic data. The 0.1 M HCL acidification results in a dirtier extract and tends to build residue in the injector port faster than the acetate buffering systems. Moreover, both 1.0 M acetate buffer and 0.1 M acetate buffer pH 4.5 saturated with NaCl demonstrate a more compacted protein layer between the aqueous and organic layers, resulting in a cleaner extraction. A cleaner extract reduces residue build up and drug decomposition in the GC injector port; thereby, minimizing routine instrument maintenance. However, the 1.0 M acetate buffer pH 4.5 assures the pH stability of blood and urine during extraction and is therefore the preferred buffering system. Chromatographic data were evaluated by comparing split, splitless, and pulsed-split modes. Split and pulsed-split modes offer improved peak symmetry and less column overload. In addition, calibration curves were linear from 4-60 mg/L with R^2 values of 0.995 for carisoprodol and 0.999 for meprobamate. The extraction efficiencies were 48% (barbital), 69% (carisoprodol), and 71% (meprobamate). Thus, 1.0 M acetate buffer pH 4.5 is the optimal buffering system to provide clean extracts with consistent recoveries.

This extraction procedure provides a rapid, clean, and effective method suitable for detecting carisoprodol and meprobamate with the intended purpose of providing analytical data to determine drug concentrations in suspected DUI cases.

References:

1. Journal of Forensic Science 2000; 45(3):619-23
2. Journal of Analytical Toxicology 2006; 30(5):302-5

Carisoprodol, Meprobamate, GC/MS

K16 Issues Pertaining to the Analysis of Buprenorphine and its Metabolites by GC-MS

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After attending this presentation, attendees will better understand the low-cost and widely available GC-MS technology can be effectively applied to the analysis of buprenorphine (B) and its metabolites in urine specimens.

This presentation will impact the forensic community by reporting the following issues pertaining to the analysis of B and its metabolites by GC-MS: (a) selection of extraction methods for the determination of free and total B and norbuprenorphine (NB); (b) effectiveness of hydrolysis, derivatization, and internal standard; and (c) deriving the contents of the glucuronides based on the free and total concentrations of B and NB observed from a two-step analytical protocol.

"Substitution therapy" and the use of B as an agent for treating heroin addiction continue to gain acceptance and have recently been implemented in Taiwan. Mature and widely utilized GC-MS technology can complement the low-cost and highly sensitive immunoassay (IA) approach to facilitate the implementation of analytical tasks supporting compliance monitoring and pharmacokinetic/pharmacodynamic studies. Issues critical to GC-MS analysis of B and NB (free and as glucuronides), including extraction, hydrolysis, derivatization, and internal standard, are studied, followed by comparing the resulting data against those derived from IA and liquid chromatography-tandem mass spectrometry methods. Commercial solid-phase extraction devices, highly effective for recovering all metabolites, may not be suitable for the analysis of free B and NB; acetyl-derivatization products exhibit the most favorable chromatographic, ion intensity, and cross-contribution characteristics for GC-MS analysis; B-d_4 can effectively serve as the single internal standard for the quantitations of both B and NB. The 2-aliquot GC-MS protocol hereby developed is proven effective for the analysis of free B and NB and their glucuronides.

Buprenorphine, Glucuronide, GC-MS

K17 GC/MS Method Development for the Quantitation of Quetiapine in Various Biological Specimens

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After attending this presentation, attendees will be aware of a GC-MS method that can be used to detect and quantitate the presence of quetiapine in biological specimens using chemicals and instrumentation that is widely available in most laboratories.

This presentation will impact the forensic community by providing a new method for the detection of quetiapine.

Quetiapine ($C_{21}H_{25}N_3O_2S$) is classified as a dibenzothiazepine derivative and is used clinically as an antipsychotic for the treatment of schizophrenia and bipolar disorder. In the body, quetiapine acts as an antagonist, targeting the serotonin and dopamine receptors. Quetiapine is metabolized in the liver, with 73% eliminated through the urine. Less than 1% of the parent drug is eliminated unchanged. Quetiapine is known to be 83% plasma protein bound and have a volume of distribution of 10 ± 4 L/kg. It is administered orally as a fumarate salt in 25mg, 100mg, 200mg, 300mg, and 400mg tablets. The fumarate salt is comprised of two quetiapine molecules per one fumarate molecule (MW = 883.1). The drug is structurally similar to the antipsychotic drug clozapine.

The Montgomery County Coroner's Office (MCCO) encountered quetiapine in 47 cases in 2007. Incidents of quetiapine in casework are increasing and as MCCO did not possess a method for the quantitation of quetiapine, specimens had to be analyzed by an outside laboratory. A study was completed identifying an extraction and instrumental procedure for the detection and quantitation of quetiapine in order to diminish the cost of outside testing. The postmortem specimens analyzed were blood, brain, liver, cerebral spinal fluid, bile, vitreous fluid, and urine. The internal standard was Smith Kline French-525A (SKF-525A). Calibrators were prepared from a quetiapine stock standard solution at concentrations of 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1.0, 1.5, and 2.0µg/mL. Liquid and powder forms of quetiapine were prepared for controls. The analysis was completed by following an in-house liquid-liquid extraction for basic drugs. Quetiapine was extracted with hexane/isoamyl alcohol (99/1). Back extraction was completed by the addition of hydrochloric acid. The drug was re-extracted into methylene chloride, which was then evaporated to dryness. Derivatization was completed by reconstituting with BSTFA + 1% TMCS and heating for 20 minutes at 75ºC. One microliter was injected on an Agilent 5973 Series gas chromatograph mass spectrometer (GC-MS) with a DB-5MS (30m x 0.25mm x 0.25µm) column. The temperature program has an initial temperature of 100ºC with an increase of 20ºC per minute to a final temperature of 285ºC. Single ionization mode (SIM) was used with the quetiapine target quantitation ion 210 and the qualifier ions 239 and 321. The target quantitation ion for the internal standard was 86. The assay was linear from 0.01 - 2.0µg/mL.

Quetiapine was identified to have a retention time of 22.64 minutes. Linear regression analysis indicated an R^2 value of 0.9952 over the entire calibration range. The concentration range of quetiapine in twelve blood specimens was 0.16 – 1.75µg/mL. The postmortem distribution of quetiapine in all other specimens were as follows: brain 0.10-1.90µg/mL (6 cases), liver 0.14-1.69µg/mL (6 cases), cerebral spinal fluid 0.10-0.18µg/mL (3 cases), bile 0.10-0.64µg/mL (3 cases), vitreous fluid 0.12-1.42µg/mL (4 cases), and urine 0.01-0.77µg/mL (7 cases). The completion of this study identifies a method that MCCO can utilize to detect and quantitate quetiapine.

Quetiapine, Postmortem Toxicology, Gas Chromatograph Mass Spectrometry

K18 Postmortem Analysis of Buprenorphine/ Norbuprenorphine From Whole Blood by GC/MS

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After attending this presentation, attendees will understand the general principles of burprenorphine, the prevalence and use of the drug in society, and the importance of analyzing for it at the Los Angeles County Department of Coroner.

The presentation will impact the forensic community by providing information on how to extract Buprenorphine and Norbuprenorphine from postmortem specimens with detection by gas chromatography/mass spectrometry (GC/MS).

Buprenorphine, is a semi-synthetic chemical derivative of thebaine

which is used to relieve moderate to severe pain. As of 2002, the FDA approved the use of buprenorphine tablets for treatment of opioid addiction. The number of cases seen at the Los Angeles County Department of Coroner involving buprenorphine has slowly increased in the past few years because of their use in addiction clinics. The current literature describes methods for buprenorphine detection in various matrices such as hair, urine, and whole blood by LC/MS/MS; however, most do not require a comprehensive sample preparation necessary for GC/MS detection. Given the fact that most forensic toxicology laboratories are equipped with the GC/MS rather than the LC/MS/MS the object of this study was to develop and validate a method for the extraction of buprenorphine and its active metabolite, norbuprenorphine from postmortem blood with detection by GC/MS. The analysis consisted of a protein precipitation with acetonitrile, solid phase extraction, and silylation derivitazation with MSTFA. Quantitation was performed with the use of deuterated internal standards, d4-Buprenorphine and d3-Norbuprenorpine and the instrument was operated in the selected ion monitor (SIM) mode with the following ions:

d4-Buprenorphine	Buprenorphine	d3-Norbuprenorphine	Norbuprenorphine
454 *	450 *	527 *	524 *
486	482	509	506
510	506	528	525

* Quantitation ion

Linearity was achieved over a concentration range of 2.0 – 25 ng/ml for both drugs supplemented in porcine whole blood with a correlation coefficient exceeding 0.99. The percent recovery of buprenorphine (83%) and norbuprenorphine (68%) was determined at three concentrations (2.0, 5.0, and 10 ng/ml) over four separate days. Limit of quantitation was 2.0 ng/ml and the upper limit of linearity (beyond 25 ng/ml) was not explored as casework would be repeated at a dilution to be within the curve. The intra-assay reproducibility (n=4) was determined for buprenorphine 2.0 ng/ml (CV 11.53%), 5.0 ng/ml (CV 7.86%), 10 ng/ml (CV 4.81%) and norbuprenorphine 2.0 ng/ml (CV 11.78%), 5.0 ng/ml (CV 7.93%), and 10 ng/ml (CV 4.73%). The inter-assay reproducibility (n=12) was determined for buprenorphine 2.0 ng/ml (CV 8.30%), 5.0 ng/ml (CV 7.05%), and 10 ng/ml (CV 4.37%) and norbuprenorphine 2.0 ng/ml (CV 9.47%), 5.0 ng/ml (CV 7.74%), and 10 ng/ml (CV 4.05%). The method was determined to be free from matrix interferences (liver, bile, and urine) by the supplementation of buprenorphine and norbuprenorphine, in duplicate, at 10 ng/ml. Quantitation of both drugs were not affected by any matrix when compared with a blood calibration curve; however, the recovery of norbuprenorphine was severely diminished in the liver specimen, whereas, all the others had no effect. Lastly, the method was successfully verified by the comparison of three different external controls as well as casework that had previously been outsourced.

Buprenorphine is increasing in popularity both on the streets as well as being used in addiction clinics. Therefore, the analysis of buprenorphine and metabolite needs to be a common practice amongst postmortem toxicology laboratories.

Buprenorphine, Analysis, Postmortem

K19 A 5-Year Stability Study on Phencyclidine and Zolpidem in Postmortem Blood Samples

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The goal of this presentation is to evaluate the stability for both phencyclidine and zolpidem over a 5-year time period in postmortem blood samples.

This presentation will impact the forensic community by demonstrating the effects of storage conditions and time on postmortem blood samples containing either phencyclidine or zolpidem and contributing additional knowledge to the proper interpretation of reanalyzed samples containing these drugs.

Phencyclidine, or PCP, is a dissociative anesthetic but exhibits stimulant, depressant, hallucinogenic, and analgesic properties. Phencyclidine is an illicit, Schedule II drug. Zolpidem is a sedative-hypnotic, Schedule IV drug, classified as a derivative of the imidazopyridines. Zolpidem is available by prescription and is used in the treatment of short-term insomnia. Due to the abuse of phencyclidine and the increase in popularity and overdose potential of zolpidem, it is essential to show how stable these drugs are in postmortem samples.

The stability for drugs in postmortem samples is extremely critical in establishing the validity of scientific results. Stability for the analyzed drug should be considered in order to justify the precision of the analytical method and the reliability of the results. Factors that may influence drug stability in stored samples include: storage temperature, storage time, addition of preservatives, and initial condition of the collected sample. Storage conditions may vary depending on the analyte of interest. This study will test the research hypothesis of whether drug concentrations for either phencyclidine or zolpidem remain stable, when samples are frozen at –20°C for up to five years. The quantitative examination for stability of 23 positive phencyclidine cases and 26 positive zolpidem cases from the Maricopa County Office of the Medical Examiner (OCME) is presented here.

This study re-analyzes postmortem blood samples quantitatively for any changes in concentration of phencyclidine and zolpidem, over 5 years. The postmortem blood samples were collected at autopsy, preserved with sodium fluoride, and stored at 4°C until initially analyzed. After the analysis the samples were stored frozen at –20°C until the samples were reanalyzed for this study. The methods of quantitation used in the re-analysis study are the same methods used when initially quantitated. For phencyclidine (n=23) liquid-liquid extraction is used followed by quantitation by gas chromatography/mass spectrometry (GC/MS). For zolpidem (n=26) liquid-liquid extraction is used followed by quantitation by gas chromatography with a nitrogen-phosphorous detector (GC-NPD).

The results obtained for phencyclidine show a tendency for concentrations to decrease over a period of 5 years. Table 1 shows the initial and final concentration ranges obtained (reported to two significant figures), along with the average decreases observed for samples stored for 5 years.

Table 1. Phencyclidine Concentration Changes

Storage Time (Since Collection)	N	Initial Concentration Range (mg/L)	Final Concentration Range (mg/L)	Percent Average Decrease
±1 year	8	(0.01 – 0.22)	(<0.01 - 0.20)	10.9%
±2 years	5	(0.03 – 0.11)	(0.02 – 0.10)	8.6%
±3 years	3	(0.09 – 0.23)	(0.07 – 0.18)	21.5%
±4 years	3	(0.02 – 0.14)	(0.01 – 0.13)	18.8%
+5 years	4	(0.04 – 0.73)	(0.03 – 0.48)	20.3%

For the phencyclidine cases (n=23), there were 12 cases showing a decrease of 10% or more, of which 9 of these cases had a decrease of 20% or more. The results indicate that phencyclidine remains sufficiently stable to be detected within 5 years of storage at 4°C, then –20°C. However, there is a significant decrease in concentration after 3 years of storage at 4°C, then –20°C.

The results obtained for zolpidem show a tendency for concentrations to both increase and decrease over a period of 5 years. Table 2 shows the initial and final concentration ranges obtained (reported to two significant figures), along with the average decreases and increases observed for samples stored for 5 years.

Table 2. Zolpidem Concentration Changes

Storage Time (Since Collection)	N	Initial Concentration Range (mg/L)	Final Concentration Range (mg/L)	Percent Average Decrease	Percent Average Increase
±1 year	12	(0.06 – 34.44)	(0.07 – 34.60)	24.1% (n=5)	12.6% (n=7)
±2 years	8	(0.15 – 0.97)	(0.15 – 0.80)	16.1% (n=3)	10.1% (n=5)
±3 years	2	(0.29 – 3.39)	(0.20 – 3.46)	29.6% (n=1)	2.3% (n=1)
±4 years	2	(1.20 – 8.42)	(0.73 – 5.20)	38.6% (n=2)	-
+5 years	2	(0.10 – 0.28)	(0.09 – 0.23)	9.5% (n=2)	-

For the zolpidem cases (n=26), there were 11 cases showing a decrease of 10% or more, of which 4 of these cases had a decrease of 20% or more. The results also show that for 7 cases the concentrations increased 10% or more over time, of which 3 of these cases had a 20% or more increase. The results indicate that zolpidem remains sufficiently stable to be detected within 5 years of storage at 4°C, then –20°C. However, there is a significant decrease in concentration within 1-year of storage at 4°C, then –20°C.

Stability, Phencyclidine, Zolpidem

K20 Method Development for the Analysis of Non-Traditional Drugs Used to Facilitate Sexual Assaults

*Jennifer L. Greaux, BS**, and Bruce R. McCord, PhD, Department of Chemistry, Florida International University, University Park, Miami, FL 33199*

After attending this presentation, attendees will become aware of a wide array of "non-traditional" drugs which have the potential to be used to facilitate sexual assaults. In addition, attendees will gain insight into the use of capillary electrophoresis (CE) for drug analysis and the advantages and disadvantages of using such a technique when coupled to a UV detector and an electro spray ionization time-of-flight mass spectrometer (ESI-TOF-MS).

This presentation will impact the forensic community by providing a more efficient technique for drug analysis and introducing new methodology for analyzing "non-traditional" drugs which have the potential to be used to facilitate sexual assaults.

The term drug-facilitated sexual assault (DFSA) has been assigned to cases where a drug(s) has been used to incapacitate an individual so that he/she is unable to consent to sexual activity.

The overall purpose of this project was to develop and optimize methods for the analysis of drugs which may be found in blood and urine specimens from sexual assault cases. It was desirable that these methods also provide accurate identification and confirmation when compared to standards. Drug standards have been prepared at various concentrations in buffer and deionized water and separated using CE-UV and CE-MS. These mixtures were comprised mainly of drugs belonging to the following classes: anticholinergic, anticonvulsant, antidepressants, antihistamines, antihypertensive, cough suppressants, and muscle relaxants. The compounds selected have been identified as candidates for DFSA because they may cause sedation, amnesia, and lower an individual's ability to resist a sexual assault.

Some of the problems surrounding sexual assault samples are that there is a limited time window for detection and that the drugs may have widely varying chemical properties and may be present in very low concentrations. Therefore, a technique is required that is fast, efficient, and very sensitive for DFSA samples. It is proposed that CE coupled to MS may be a useful technique to analyze these compounds due to its high resolution and wide range of sample detection capabilities. In addition, the application of time of flight mass spectrometry greatly improves the ability to detect and identify unknown analytes. Due to its high (3ppm) resolution, the time of flight system permits infusion of samples prior to separation as a quick and efficient prescreening tool.

Optimization of developed methods was performed by altering parameters such as buffer pH and concentration, voltage, and sample injection. Additionally, the effects of adding organic modifiers and a water plug were examined. Phosphate buffer at low pH was used as the run buffer as it will cause the drugs to remain charged and suppress the electroosmotic flow to allow sufficient time for separation. The limits of detection and reproducibility of results were also evaluated to determine the relevance of this study to "real-life" samples.

The analysis of various drug mixtures will be detailed to show that capillary electrophoresis is an efficient and reliable technique for drug detection of sexual assault samples. Such a technique can then be used to aid authorities in prosecuting criminals accused of sexual assault in a quick but efficient manner.

Capillary Electrophoresis, DFSA, Method Development

K21 Screening of Anabolic Steroids in Suspected DUI Drivers in Miami - Dade Florida Using ELISA Kits

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After attending this presentation, attendees will better understand the possible role that steroids may play in suspected DUI drivers. The goal of this presentation is to suggest a reliable screening method for the steroids boldenone and stanozole in biological samples.

This presentation will impact the forensic community by providing information on a reliable methodology for the screening of steroids in urine and blood and establishing the incidence of steroid abuse in the suspected DUI community of Miami-Dade County. This information is important to establish possible drug abuse patterns in our communities and help identify possible causation factors for suspected drug impaired driving cases.

Anabolic steroids such as boldenone and stanozolol are compounds related to testosterone. Steroids are reported as being abused by professional athletes to increase strength and muscle mass; however, there are reports of abuse amongst the general population. It is understood that high doses of anabolic steroids can cause aggressive behavior, insomnia, and irritability. Anabolic steroids have been also reported to cause other behavioral effects, including euphoria, increased energy, sexual arousal, mood swings, distractibility, forgetfulness, and confusion. These reported side effects may have an effect on driving skills and therefore may be compounds of interest in suspected DUI drivers.

The purpose of this study was to evaluate the occurrence, if any, of boldenone and stanozolol in suspected DUI drivers in Miami-Dade County. If a sample is recorded as positive then the "Drug Recognition Expert" evaluation was examined to correlate symptoms with drug use.

Blood and urine samples were submitted to the forensic toxicology

lab for drug and alcohol screening. Boldenon and stanozolol were screened for in blood and urine by ELISA kits for all samples received in 2008. In addition, all blood and urine samples over the past 5 years that were negative in routine drug screens were analyzed. If positive, steroids were qualified and quantified in blood/urine by GC-MS. Case histories, including the DRE evaluation were collected and positive results were evaluated using this information.

Providing information on possible steroid abuse may explain some behavior and impairment seen in suspected DUI drivers when all other toxicological screening is negative. This information is important to determine potential drug abuse in our community and help identify possible causation factors for suspected drug impaired driving cases.

Anabolic Steroids, ELISA, Driving

K22 Rapid Inline Derivatization of Primary and Secondary Amine Containing Drugs Using NBD-F and CE-LIF

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After attending this presentation, attendees will be able to understand the mechanism by which drugs containing primary and secondary amine groups are derivatized on — capillary using NBD — F for the purpose of detection using capillary electrophoresis with laser-induced fluorescence.

This presentation will impact the forensic community by explaining how this method allows for the sensitive and rapid detection of drugs in bodily fluids for the purpose trace analysis and general drug screening.

Capillary electrophoresis has become an increasingly common analytical method in forensics due to its flexibility and the wide variety of detection systems which can be used. One particularly useful method of detection in CE is laser-induced fluorescence, LIF. The application of LIF permits highly sensitive detection of compounds using CE in spite of the narrow pathlength inherent in the procedure. However, the number of compounds in which fluorescence occurs naturally are few and in order for most compounds to fluoresce, derivatization is necessary.

There are a variety of fluorescent dyes which can be coupled to primary and secondary amines. Derivatization can take place through reactions with dyes linked to reactive groups such as isothiocyanates, succinamidyl esters, and other amine reactive groups. These derivatizations can be performed before the analyte enters the capillary, while it is on the capillary during the separation or after the separation has been completed, post-capillary. On-capillary derivatization is not used as commonly as pre-capillary or post-capillary derivatization due to difficulties in reproducibility and optimization of the derivative yield. If these issues could be overcome it would greatly increase the throughput of analyses given and permit the use of inline, microfluidic techniques.

A feature common to many drugs of abuse are primary, secondary, or tertiary amine moieties. NBD-F is a non-fluorescent compound which reacts to primary and secondary amines by losing the fluorine attached to the benzene ring and joining to the analyte at the nitrogen which loses a hydrogen atom. The resulting derivative is strongly fluorescent and has an emission wavelength around 530 nm. Given that prior to derivatization NBD-F is not fluorescent, the excess reagent produces minimal interference with the analyte permitting sensitive and specific detection of the drug conjugates. The overall process permits a highly sensitive and rapid screen for drugs in body fluids.

This paper will discuss the development of in-line derivatization techniques for trace detection and screening of phenethylamines and other drugs of abuse.

On-Capillary Derivatization, Phenethylamines, Capillary Electrophoresis

K23 Importance of Postmortem Adipose Tissue Analysis in an Olanzapine (Zyprexa) Suicide Case

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The goal of this presentation is to suggest the value of adipose tissue analysis for the identification of drug users is steadily gaining recognition. Adipose tissue analysis may be useful adjunct to conventional drug testing in toxicology. Specimens can be more easily obtained with less embarrassment and adipose tissue can provide a more accurate history of drug use. After attending this presentation, attendees will understand the importance of biological alternative matrices in postmortem toxicological analysis.

This presentation will impact the forensic community by demonstrating the utility of adipose tissue analysis in determining defensible cause of death to evaluate the pharmacological story. The objective of this presentation is to provide long-term information about an individual's drugs use, especially when the pharmacological history is difficult or impossible to obtain. A sensitive and specific GC/MS method for the determination of drugs in postmortem adipose tissue was used. The method combines acid extraction of analytes, alkalinization of the extract aqueous, purification on Extrelut NT columns and GC-MS analysis.

This case involves a 22-year old female who suffered from depression and was on benzodiazepines and antipsychotics: lorazepam, valproic acid, chlorpromazine, and sertraline. There was history of three previous attempted suicides. At the crime scene, a large number of antidepressants, antipsychotics and benzodiazepines packs (some of which were empty), and an empty olanzapine (Zyprexa) pack. Systematic toxicological analysis was performed on conventional biological samples for drug of abuse, alcohol, and other poisons. Urine immunochemical screening and GC/MS analysis detected all drugs prescribed, in therapeutic concentrations (lorazepam, valproic acid, chlorpromazine, and sertraline). Blood immunochemical screening and GC/MS analysis detected all drugs prescribed in therapeutic concentrations and a olanzapine (Zyprexa) concentration of 3.07µg/ml, greater than the therapeutic concentration range of 0.01- 0.05 µg/ml.

Toxicological analysis on adipose tissue confirmed the presence of all drugs prescribed (Lorazepam, valproic acid, chlorpromazine, and sertraline) and found at the crime scene, but did not reveal the olanzapine presence. The large presence of olanzapine, not prescribed drug, in the blood and not in adipose tissue is indicative of the olanzapine intake for suicide. Therefore, the death was ruled a suicide caused by olanzapine overdose. In conclusion, this study suggests the value of adipose tissue analysis for the identification of drug users and is steadily gaining recognition. Adipose tissue analysis may be useful adjunct to conventional drug testing in toxicology. Specimens can be more easily obtained with less embarrassment and adipose tissue can provide a more accurate history of drug use.

Adipose Tissue Analysis, Olanzapine, Suicide

K24 Impact of Drugs and Alcohol on Manner of

Death by Sex and Age Among Autopsy Cases Performed at the Upper East Tennessee Forensic Center in 2007

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After attending this presentation, attendees will understand of the impact of drugs and/or alcohol on the manner of death by sex and age among autopsies performed at the Upper East Tennessee Forensic Center in 2007.

This presentation will impact the forensic community by illustrating the increased impact of drugs and alcohol on the manner of death in a select region of Tennessee.

The Upper East Tennessee Forensic Center performs autopsies on the questionable and medicolegal deaths which occur in the eight counties of the First Tennessee Development District. Toxicological evaluations of specimens collected at autopsy are used to determine if drugs and/or alcohol are involved in determining the cause and manner of death. A descriptive database was established defining all parameters and data pertinent in each case (age, sex, cause/manner of death, and toxicological results). The purpose of this research was to determine descriptive statistics on the impact of drugs and/or alcohol by manner/cause of death, age, and sex in the autopsies performed in 2007. Specimens (blood, gastric contents, urine, vitreous humor, and bile) from the autopsies were analyzed for drugs and alcohol using multiple analytical toxicological procedures (colorimetric, TLC, immunochemistry, GC, GCMS, and LCMS). Toxicological results were compiled in an electronic database to allow for analysis and interpretation. Results indicate that out of 277 total cases, 66% were male, 34% were female, 85% were positive for drugs, 27% were positive for alcohol, 23% were positive for both drugs and alcohol, and 12% had neither drugs nor alcohol. Analysis of the distribution of cases positive for drugs, alcohol, and drugs/alcohol revealed that males had a greater percentage of cases involving alcohol alone as well as cases positive for drugs/alcohol than females. Acute drug overdoses accounted for 34% of total cases with no substantial sexual differentiation. Of the 94 acute overdose cases, 4 (»4%) were intentional (suicides) and 90 (»96%) were accidental. Autopsies were performed on all age groups (percentage of cases/ years of age): 5% <14, 4% between 15 and 19, 8% between 20 and 24, 14% between 25 and 34, 22% between 35 and 44, 16% between 45 and 49, 13% between 50 and 54, 10% between 55 and 64, 6% between 65 and 74, and 2% ³75 years of age. The distribution of positive drug cases closely mirrored the distribution of cases by age groups. Manner of death analysis revealed (of total cases) that 47% were accidental, 27% were natural, 15% were suicides 6% were homicides, and 5% were undetermined. No appreciable disparity in distribution of manner of death was found between the sexes. Analysis of the results of the toxicological evaluations revealed there were a large number of cases in which opiates (100), alcohol (75), benzodiazepines (110), sedatives (18), and/or stimulants (14) were identified. Review of these results leads to the conclusion that drugs and alcohol have a significant impact in the questionable and medicolegal deaths occurring in Upper East Tennessee.

Drugs, Alcohol, Death

K25 Relationship Between Drug Levels and the Causes and Manners of Death in Methamphetamine Related Casualties: A Retrospective Study

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After attending this presentation, attendees will have knowledge of methamphetamine blood/urine levels distribution in the manners of death with a retrospective review cases for seventeen years.

This presentation will impact the forensic science community by utilization of the toxicological profiles in the determination of forensic parameters including the cause and manner of death, especially for methamphetamine related fatalities.

Methamphetamine (MAP), an illicit, stimulant drug, has resulted in serious social problems in Taiwan and other parts of the world. A pilot study was designed to determine whether toxicological profiles of decedents' body fluids can be used to implicate the status of mood at the moment of death. High blood/urine ratios can be associated with acute MAP use, shortly after MAP intake and a manic emotional status. In comparison, a low blood/urine ratio can be associated with chronic MAP use, after a longer period of time following MAP intake and a depressive emotional status. This retrospective review of 18,973 fatalities collected from Institute of Forensic Medicine in Taiwan from 1991 to 2007. MAP levels both in blood and urine that were greater than 0.02 mg/L and with positive impressions of the causes and manners of death were found in 212 cases. Distinct patterns of MAP levels were distinguished to be associated with manner or pattern of death.

Higher MAP concentrations were found in blood than in urine when death occurred shortly after an overdose of MAP that was linked either to accidental overdose (3.24 ± 0.73 mg/L blood, 15.08 ± 2.38 mg/L urine and 22.07 ± 4.22 urine/blood ratio; n=88) or to intentional suicide (12.81 ± 5.30 mg/L blood, 14.68 ± 5.57 mg/L urine, and 15.38 ± 12.96 urine/blood ratio; n=7). Lower MAP blood levels and urine/blood ratios were found in cases of accidental deaths (0.31 ± 0.06 mg/L blood, 5.72 ± 1.31mg/L urine and 34.86 ± 9.81 urine/blood ratio; n=30), and suicides not related to high MAP dose (0.55 ± 0.13 mg/L blood, 10.35 ± 2.75 mg/L urine and 34.71 ± 9.65 urine/blood ratio; n=20), thus making a highly suspicion of influence of MAP mediated through depression and psychotic behaviors. Much higher MAP urine/blood ratios and lower MAP blood levels were found among casualties of natural causes (0.40 ± 0.09 mg/L blood, 14.88 ± 4.60 mg/L urine, and 81.07 ± 44.86 urine/blood ratio; n=19) or homicidal causes (1.26 ± 0.19 mg/L blood, 13.19 ± 1.95 mg/L urine, and 16.66 ± 2.80 urine/blood ratio; n=48), suggesting these were relatively unaffected by the lower blood level of MAP. Chronic MAP abusers appear to provoke violent behaviors resulting in the homicidal fatalities, and relationship to amphetamine (AMP)-like psychosis is postulated.

These results suggest that the toxicological profile of MAP concentrations in blood and urine can play a crucial role and are related better to patterns of death than manner of death. The findings may enable better utilization of the toxicological profiles in the determination of forensic parameters including the cause and manner of death in MAP

related fatalities.

Methamphetamine, Manner of Death, Cause of Death

K26 An Unusual Case of Ethanol/Methanol Poisoning: Or Was It? The Million Dollar Question!

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After attending this presentation, attendees will be able to: (1) Recognize common problems that can confound the correct interpretation of blood ethanol determinations, (2) Identify issues that can reduce the reliability of postmortem blood ethanol or methanol test results, and (3) Develop a set of questions which should be addressed regarding the integrity of any blood sample obtained from the living or deceased.

This presentation will impact the forensic community by explaining the confounding issues in the interpretation of blood ethanol test results can be reduced by obtaining the answers to several probative questions regarding person, site, and methods of blood drawing and storage.

Common problems can confound the correct interpretation of typical blood ethanol or drug tests, especially when the blood has been taken from a dead body. The presented case is an example of some of the difficulties that can be encountered, and provides a set of questions which should be addressed about the integrity of any blood sample, but most importantly, a blood sample that has been obtained from a dead body.

A professional truck driver in Alaska was found dead in the cab of his truck after it was driven off the road and had rolled down a hill. Three empty beer cans and a sandwich wrapper were found in the cab behind the driver. Toxicology analyses from a certified laboratory reported a blood ethanol level of 0.086% (single value) and a blood methanol level of 0.15%. If the driver was impaired at the time of the accident, worker's compensation would not pay death benefits to the decedent's wife and family. However, if impairment was not proven, then the family would receive insurance and death benefits.

The insurance company claimed the driver had been impaired and retained a forensic pathologist who reviewed the laboratory tests and signed an affidavit stating that the decedent had ingested both ethanol and methanol (from Sterno) prior to death and had been impaired at the time the truck rolled down the hill. This author was retained by the attorney for the widow and the family to investigate the circumstances of the ethanol and methanol blood analyses.

When the attorney called, I asked, "Who drew the blood sample?" He responded, "I don't know." I asked, "Where was the blood sample drawn, in the hospital?" "No, it was drawn in a mortuary." This answer provided the critical information to infer that the sample was unreliable, and, knowing that embalming fluid contained both formaldehyde and methanol, that the blood sample most likely was obtained after the body had been embalmed.

The rest of the case was easy. Take the mortician's deposition, determine the body site from which the sample was obtained, discover the name of the company that supplied the chemicals used to embalm the body, and get copies of the Material Safety Data Sheets (MSDS) to ascertain the chemical composition of the embalming fluids. The deposition also indicated that the mortician had not obtained a true blood sample. Instead, he had found a small collection of blood-tinged fluid in the body cavity and had submitted that for testing labeled as "blood." The MSDS for one of the two chemicals used to embalm the body stated that the product contained 20% methanol.

When blood samples are obtained in a hospital or by law enforcement, appropriate procedures are followed in order to assure the sterility and integrity of the sample in order to conform to existing standards and ensure the reliability of the results. When blood samples are obtained from dead bodies, often the sterility of the sample cannot be assured, and contamination of the sample by bacteria can lead to the production of postmortem ethanol both *in situ* and *in vitro* (Zumwalt et al, 1982), which can lead to unreliable results. Bacterial degradation and metabolism of endogenous substances like glucose (Clark et al, 1982), lactate (Bogusz et al, 1970), glycerol (from fat), and amino acids (Corry, 1977) also have been shown to produce ethanol in dead bodies.

While collecting and analyzing blood, other body fluids like vitreous humor and urine (Levine et al, 1993) can be helpful in determining the source of the ethanol in blood, and asking a few simple questions about the acquisition of the blood sample also can be illuminating. Such questions include: Who drew the blood sample? Who performed the analysis? What were the qualifications of the operator? From what anatomical site was the blood sample obtained? Was a preservative like sodium fluoride or sodium azide used? Was an anticoagulant like EDTA, Ca oxalate, Ca citrate, or heparin used? Was the blood "spun down" before storing? Was there any hemolysis present in the sample? Under what conditions was the sample stored? How much time elapsed between sample drawing and analysis? Which laboratory did the testing? For ethanol, was the testing done with a non-specific Alcohol Dehydrogenase assay that measures NADH production or other screening-level test, or by gas chromatography? What was the specificity of the test procedure? What was the sensitivity of the test procedure? Was the sample collected, transported and processed under a Chain of Custody? Was a "test kit" used or a laboratory protocol? If so, can you get a copy of the manufacturer's labeling or the laboratory's protocol? Only questions provide answers.

Integrity of a Blood Sample, Reliability of Test Results, Solving a Case

K27 Exsanguinating Hemorrhage From a Ruptured Gravid Uterus Resulting in Maternal and Near Full Term Infant Death Following Cocaine Abuse

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After attending this presentation, attendees will gain an understanding of some of the complications associated with heavy cocaine use during the late stages of pregnancy. Specifically, this presentation addresses the postmortem analytical results of a mother and her in-utero fetus that died following uterine rupture after a period of cocaine use. This information may be applicable to previous, current or future cases that involve similar circumstances, whether the case leads to the death of the fetus, mother, or both.

This presentation will impact the forensic community by providing pharmacokinetic data for a case in which limited data are currently available. This presentation describes to the forensic sciences community a case that involves late term pregnancy, cocaine use, and the problems encountered by both mother and fetus. While previous studies have shown that cocaine use during pregnancy impacts the uterus' vasculature, as well as the overall health of mother and fetus, the exact anatomical and physiological impacts have not been determined. The demand for focus on cocaine use by pregnant women has been steadily increasing since the 1980's. While the effects of cocaine on the average

person are better understood, the drug's effects on a developing fetus and the uterine structure are less evident. This case may help to define the distribution of cocaine between mother and fetus during heavy cocaine use.

Case History: A 32-year-old gravid 4, para 3 mother in her 36th week gestation was found in the morning sitting on a toilet slumped to the left with her head resting on the sink. A small amount of vaginal bleeding was observed and white powder was found at the scene. Emergency personnel were called and the patient was transported to a regional medical facility. Cardiac monitoring and EKG showed sinus rhythm, but no pulse. No fetal heart tones were detected. Resuscitation efforts were terminated 2 hours later.

Analytical Results:

	Mother		Fetus		
	Cocaine	BE	Cocaine	BE	
Right Heart Blood	3,756	9,368	524	3,734	(Heart Blood)
Femoral Blood	1,470	11,648	1,996	18,120	(Umbilical)
Urine	4,674	88,006	1,159	4,643	(Kidney)
Vitreous	3,167	3,590	1,023	5,103	(Liver)

Analytical data of lung, muscle, brain, adipose, and epidermis (from skin slippage) from the fetus were also obtained.

The mechanism of death is in this case is exsanguination from the ruptured uterus through a previously thinned uterine wall. The approximate 36-week gestation female infant was partially extruded or expelled through the ruptured uterus into the peritoneal cavity. The infant died due to exsanguinating hemorrhage of the mother following rupture of the gravid uterus through the previous C-section scar. Cocaine abuse contributed to the uterine rupture and infant death.

Uterine Rupture, Cocaine, Exsanguination

K28 Collaboration of Emergency Clinician and Forensic Toxicologist in a Suicide Case Related With Amitriptyline

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After attending this presentation, attendees will take into consideration a rapid analysis of amitriptyline from the gastric lavage by High Performance Thin Layer Chromatography (HPTLC) method and confirmed by Gas Chromatography Mass Spectrometer (GC-MS) method during the treatment of a suicide case.

This presentation will impact the forensic science community by highlighting the importance of collaboration between the emergency clinician and forensic toxicologist in a suicide case related with amitriptyline.

A 32-year-old "pharmacist man" was brought to Cerrahpasa Emergency Department by relatives after 3 h ingested 29 tablets of 25 mg Amitriptyline. On presentation, he was noted to have moderate anxiety and semi-cooperative. His vital signs included a temperature of 36.7¡C, a blood pressure of 150/100 mm Hg, a pulse rate of 150 bpm, and a respiratory rate of 18 breaths/min. His oxygen saturation was 96% while breathing room air. 12-lead ECG: Sinus tachycardia. His lungs were clear to auscultation bilaterally. His cardiac examination revealed an regular, tachycardic rhythm. There was no discernible murmur. His abdomen was soft, nontender, and nondistended. On his neurologic examination, general depression of all neurologic functions, reduced muscle tone, and tendon reflexes were noted. His Glasgow Coma Score had fallen to 12/15. After the evaluation, 3000 cc serum physiologic was given via the nasogastric tube in 10 minutes. The analysis of the recovered gastric lavage was done and evaluated in Toxicology Laboratory of Istanbul University, Institute of Forensic Sciences. After gastric lavage, activated charcoal was given and $NaHCO_3$ was applied as antidote treatment. The patient was monitored to observe changes in cardiac rate and conduction and then because of alteration of consciousness and cardiac rhythm, he was admitted to Intensive Care Unit for supportive care.

In the toxicology section, the first washings of gastric lavage was extracted with ethyl acetate:heptane (1:1) in alkaline pH and the analyte was quantified by absorbance/reflectance densitometry using peak-area ratio analysis by HPTLC. Amitriptyline amount was found approximately 26.3 mg/L of gastric lavage sample. Parallel analysis with a GC-MS showed similar quantitative results. This study confirmed the retrospective data of the patient with high doses drug intake according to related articles.

In this case, results obtained by analyzing gastric lavage proved usefulness of the presented method, which represents an enough time to evaluate the epidemiology of the poisonings. Also comparative qualitative analysis between HPTLC and GCMS showed the capability of HPTLC in identifying qualitatively.

Amitriptyline, HPTLC, GC - MS

K29 Fatal Death of an 8-Year-Old Boy From an Explosion Caused by Escaping Butane: Asphyxiation or Death by Explosion?

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After attending this presentation, attendees will be briefed on the sudden death of a small boy involved in an explosion caused by escaping butane.

This presentation will impact the forensic science community by making the attendees aware of pathological and toxicological findings that clarify the cause(s) of death.

Flammable vapors of butane are capable of migrating through an area, creating a path for fire or explosion. Vapors that spread throughout an enclosed space may become flammable or explosive if they encounter an ignition source, whether it be a flame, pilot light, spark, or friction. On the other hand, butane at high concentration can cause asphyxiation as described in several accident or suicide cases.[1]

An 8-year-old boy was found dead under the wreckage of his home which collapsed after an explosion caused by escaping butane. One thousand liters of butane had been contained in a tank in the garden that was found empty. Butane was supplied to the house for domestic use such as cooking and heating. The butane leak inside the home happened during the night and was caused by the accidental turning off of the cooker.

The goal of this forensic investigation was to clarify the cause of death and identify the factors involved in the accident. In fact, considering the large amount of butane which had escaped, it was essential to determine if death was caused by asphyxiation or by

explosion.

Toxicological analyses were performed on blood, liver and fat tissues. Butane was determined in all biological samples by HS/GC-MS. Calibrations were performed in matrix in the case of blood, while the butane amount released by the tissues was estimated using a vial to which a fixed volume of butane gaseous solution had been previously added.

Autopsy findings: External examination of the body revealed burn injuries exceeding 80% of the body surface mainly localized on the left part of the face and the thorax. These injuries could not have caused the death of the boy. Internal examination showed extensive head injuries and spinal transection with C2–C3 fractures. Fractures were also revealed in the rib cage, pelvis, and in the arms. Macroscopically, hemorrhagic edemas and passive congestion were evident in lung.

Toxicological results: Analyses confirmed that the boy had inhaled butane before death. Butane was revealed in blood at a concentration of 0.78 mg/g, while after headspace extraction, liver and fat tissue released 0.96 mg/g and 0.015 mg/g of butane, respectively. However, these results, mainly those relating to the fat tissue, demonstrate that the butane concentrations, to which the boy had been exposed, were not sufficient to have caused asphyxia.

In fact, aliphatic hydrocarbons, such as butane are lipophilic so that after being taken up from the lungs into blood, they are distributed at high concentrations in lipid-rich tissues such as fat tissues, and also in liver.[1] This characteristic is also confirmed by Kow value of butane (630.96), which, being a measure of hydrophobicity, helps to understand and/or determine the fate of chemicals after exposure. Additionally, these tissue concentrations are generally lower than those reported in butane asphyxiation cases. On the other hand, its determination also in fat tissue demonstrates that the butane leak had been particularly slow because of the body distribution of the toxicant.

Pathological and toxicological findings demonstrated that death occurred after a very short time and was not caused by butane asphyxiation. The victim's injuries localized on the front side indicated that when the accident occurred the boy was standing in front of the source of explosion. Probably, the boy himself had caused gas ignition by switching on the light. However, the autopsy showed that death was not caused by burn injures but was mainly related to the injures caused by the building collapsing.

Reference:

1. Sugie H, Sasaki C, Hashimoto C, Takeshita H, Nagai T, Nakamura S, et al. Three cases of sudden death due to butane or propane gas inhalation: analysis of tissues for gas components. Forensic Sci Int 2004 Jul 16;143(2-3):211-4.

Butane, Explosion, Asphyxia

K30 An Unusual Circumstance of Internal Chemical Burn Injury: A Case Report

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After attending this presentation, attendees will understand the injuries due to a rarely reported case of chemical burns due to ingestion of nitric acid in which the history was not of an accidental but of a suicidal nature.

This presentation will impact the forensic community by helping in formulating an emergency treatment protocol.

The present case describes the macroscopic findings of vital changes seen in patient due to ingestion of nitric acid is also highlighted. Spillage of nitric acid (vitriolage) is frequently reported especially in the third-world countries, but an ingestion injury due to nitric acid injuries are seldom encountered in routine practice.

Nitric acid, also known as aqua fortis (strong water) or spirit of nitre or engraver's acid1 is a chemical important for industrial and domestic purposes. A strong acid, powerful oxidizing agent and an ability to nitrate organic material make it an essential in the production of numerous chemicals. Skin contact leads to severe burns and its vapours can cause severe acid burns to the eyes, respiratory tract, and lungs. Being a corrosive, it produces immediate pain and causes burns of mouth, throat, esophagus and abdomen, widespread gastroenteritis, and bloody diarrhoea. Blood may also be found in urine.

A 55-year-old female unable to face the problems of life, ingested an acid around 11:00 a.m. in the morning. She was brought in with complaints of pain and burning sensation and thereby was admitted to a private medical hospital around 3:00 p.m. the same afternoon. She was a known diabetic. Following admission, the patient had undergone laboratory tests which revealed red colored urine (haematuria), proteinuria, aciduria (low urine pH), and pyuria suggesting signs of poisoning and later septic shock.

Amorphous calcium oxalate crystals were also found in urine. Serum electrolytes and other routine investigations were normal. Liver function tests (LFT) showed raised liver enzymes (SGOT = 91, SGPT = 46). Peripheral smear showed a total count of 22,300 (N92 L7 M1) which is a sign of acute inflammation and perforation. This was later confirmed with an abdominal X-ray showing pneumo-peritoneum.

The patient's condition deteriorated after two hours. Arterial blood gas analysis showed acidosis with a pH of 7.1. Serum electrolytes showed variation (serum potas- and whitish tinge of teeth was seen.

Unlike sulphuric acid, when concentrated nitric acid is ingested, the tendency to produce charring of tissues and then perforation is a rare event as recorded in the present case. It may be said that acid burn injuries represent only a minute percentage of burns, but they cause a particular type of lesion in which the morbidity is high and death is certain.

This case presents as an unusual circumstance of an internal burn injury caused due to nitric acid, a rare event, made more so by being used as an agent for suicide. It is recommended that medico-legal death investigators become familiar with the internal chemical burn injuries due to nitric acid.

Chemical Burn Injury, Nitric Acid, Nitric Acid Ingestion

K31 Suicide Cases by Insulin Administration at Tarrant County Medical Examiner's Office

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After attending this presentation, attendees will be briefed on suicide cases by exogenous insulin administration and the measurement of insulin and C-peptide using a new immunoassay in postmortem blood specimen.

This presentation will impact the forensic community by validating this new immunoassay on an automated platform and establishing the normal ranges of insulin, C-peptide, and insulin/C-peptide ratio.

An immunoassay on an automated platform was validated for determination of insulin and C-peptide in postmortem blood specimens. The insulin and C-peptide assays are FDA approved clinical assays by Siemens on the ADVIA Centaur automated platform. This is a common instrument used by many clinical and hospital laboratories. All reagents

were commercially available from Siemens, including calibrators and diluents. Both assays are approved for serum specimens. The manufacturer warns that both assays may have interferences from hemolysis. Postmortem blood specimens typically have gross hemolysis and it is impossible to obtain a clean serum specimen. Thus, postmortem specimen must be pre-treated to reduce the interference enough to obtain reliable values. Dilution of the specimen is the most convenient method that may reduce this interference. Standard addition involves adding a standard in buffer to the specimen, while serial dilution adds assay buffer. Two cases were investigated so far this year. In each case, the sample is a diluted specimen of postmortem whole blood.

The normal range by the automated immunoassay for insulin is 2.6 to 25.0 mU/L and for C-peptide is 0.9 to 4.3 ng/mL. Great than 1 ratio of insulin/ C-peptide is suggestive of exogenous insulin administration.

Case #1: A 52-year-old male with a history of depression and suicide threat was found unresponsive in his parked car. Two empty boxes of humulin insulin were found in the car. The decedent's abdomen reveals numerous injection sites. The postmortem whole blood testing shows exogenous insulin overdose. Subclavian blood insulin is 595 mU/L, C-peptide is 1.66 ng/ml, the ratio of insulin/ C-peptide is 7.56.

Case # 2: A 68-year-old female with a history of diabetes, dementia, depression, and suicidal thought was found unresponsive in her bed. A ¼ empty bottle of insulin was found at home along with a note to her daughter stated, "I love you." The postmortem femoral whole blood testing shows that insulin is 106 mU/L, C-peptide is 1.00 ng/ml, the ratio of insulin/ C-peptide is 2.24.

Values provided by the automated immunoassay platform were compared to values in aliquots of the same samples analyzed by the Mayo Clinical Laboratories (MCL). Twenty samples were tested by both methods. The correlations were 0.992 and 0.996 for insulin and C-peptide, respectively; there was no statistical difference between methods for either analyte. The mean (SD), as mU/L, for insulin was 21.8 (20.6) by MCL and 22.9 (23.5) by automated immunoassay; and for C-peptide, as ng/mL, 2.35 (2.56), and 2.65 (2.99). Thus, the automated platform provided equivalent values to MCL for both analytes.

The recoveries for insulin serial dilution on specimens from both cases ranged from 86% to 115%; the buffer to specimen ratio was 1:4 to 1:16. The recoveries for C-peptide standard addition on specimens from both cases ranged from 85% to 112%; the calibrator to specimen ratio was 1:2. In both cases presented here, the insulin concentrations were too high to use standard addition, while the C-peptide concentrations were too low to use serial dilution.

For the determination of insulin in hemolyzed postmortem specimens, samples can be serially diluted for reliable concentrations. For C-peptide, standard addition can provide reliable concentrations. The dilution was sufficient to reduce the interference caused by the gross hemolysis that occurred postmortem. The above results and case studies indicate that insulin/C-peptide ratio of great than 1 is suggestive of exogenous insulin administration as the cause of death.

Suicide, Insulin, Insulin/ C-Peptide Ratio

K32 Refusing the Refusal: A Review of Texas' Mandatory Blood Draw Initiative

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The goal of this presentation is to reveal the abuse patterns of individuals who refuse to provide a specimen in a DWI investigation. Attendees will understand the levels of drugs and alcohol that are present in the refusal demographic as compared to those who provide a specimen.

This presentation will impact the forensic community by providing toxicologists, investigators, and prosecutors with the impact that the mandatory blood draw initiative has had across the State of Texas from public awareness to numbers of arrests to prosecution of intoxicated drivers. It will also provide assurance that a mandatory draw program does not improperly target innocent drivers.

It is thought that those who refuse to provide a specimen have experience with driving under the influence of drugs and/or alcohol and their results will be elevated when compared to non-refusals.

The State of Texas is among the leaders in DWI fatalities. By statute, all drivers in Texas have consented to providing a specimen of breath or blood; however, roughly half of all DWI investigations result in the driver refusing to provide a specimen. This has been viewed by the investigating agencies as paramount to hiding evidence in a criminal investigation. Although the Mandatory Blood Draw process has been questioned and challenged by defense attorneys across the State, the process has been upheld in the Court of Appeals in the State of Texas. This has paved the way for a statewide initiative to obtain a search warrant and take a blood specimen when the driver will not voluntarily provide one.

This initiative began with a small agency in North Texas and has spread throughout the State. Most agencies will target certain "peak" times such as holidays or certain weekends to carry out the Mandatory Blood Draw event. During this time media is involved to raise awareness of the event. However, there are agencies that have gone to a full time mandatory system. The process requires the coordination of law enforcement agencies, court administrators, judges, and nurses. Each must be on call throughout the period in which the mandatory event is under way so the warrant can be issued and carried out in a timely manner.

The data suggests that the alcohol concentration is higher on average for those that refuse and the prevalence of drugs is approximately the same. This correlates well with the thought that the refusal drivers have experience with driving under the influence. Statewide alcohol and drug results will be presented comparing historical data from voluntary submissions with these new mandatory specimen results. Arrest statistics, prosecution statistics, and DWI in non-mandatory situations will also be discussed.

DWI, Mandatory, Blood

K33 A Retrospective Study of Drug Prevalence in Alcohol Related Driving Arrests

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The goal of this presentation is to help illustrate some of the difficulties involved in assessing the full impact of drug impaired driving. Officers may not be adequately trained to recognize the symptoms of drug impaired drivers, laboratories may not be performing comprehensive enough testing to identify the drugs that can impair, and prosecutors may not be pursuing the prosecution of drug impaired drivers.

This presentation will impact the forensic science community by providing data to support increased commitment and resources to combat the drug impaired driving problem.

Impaired driving investigations constitute the majority of cases submitted to the NYSP Crime Laboratory's Toxicology section. Both blood and urine are submitted, and the scope of testing is determined by the charges involved and the submitter's request. Alcohol testing is performed by headspace gas chromatography. Drug testing involves an immunoassay screen and a basic drug screen/confirmation by GC-NPD

and GC-MS, plus drug class specific confirmations as warranted. Specimens are retained at least eighteen months after analysis.

A retrospective study was conducted on blood specimens that had exceeded the eighteen month retention time. Over 300 samples from 2005 and 2006 were tested. The samples were screened by ELISA for the following eight drugs/drug classes at the indicated cut-offs: cannabinoids (10 ng/mL), cocaine/benzoylecgonine (50 ng/mL), opiates (20 ng/mL), benzodiazepines (50 ng/mL), methamphetamine/MDMA (20 ng/mL), methadone (50 ng/mL), carisoprodol (500 ng/mL), and zolpidem (25 ng/mL).

The study involved cases that had only alcohol testing performed originally. The first set of samples involved charges/requests related only to alcohol. The subsequent ELISA drug testing conducted in this study revealed 39% were presumptive positive for one or more drug/drug class. The majority, 30% of the total cases, were presumptive positive for cannabinoids. The second set of cases involved charges/requests for alcohol and drugs, but only alcohol testing was performed. Laboratory policy dictates that alcohol testing be conducted first. If the result is ≥0.11% by weight, results are reported with a statement indicating that if drug testing is still needed, you must contact the laboratory. The additional testing was not requested for these cases. However, when ELISA drug screening was performed for this study, 48% of these cases were presumptive positive for one or more drug/drug class, primarily cannabinoids.

An additional part of this study involved cases with charges/requests related to drugs, where drug testing was originally performed (some may have also included alcohol testing with results < 0.11% by weight). Since the original testing of these cases, the protocol for immunoassay drug screening in the laboratory changed from FPIA to ELISA, more assays were included, and many cutoffs were lowered. These cases were included in the study to assess the significance of these changes.

Drugs, Driving, Cannabinoids

K34 PCP and Drug Impaired Driving in San Francisco, California

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After attending this presentation, attendees will become aware of the effects that phencyclidine has on driving skills as demonstrated by case examples from drivers arrested for DUID by PCP in a three year period.

This presentation will impact the forensic science community by offering a set of reference data on PCP concentrations often measured in impaired drivers and epidemiological data of signs and symptoms associated with PCP intoxication and impairment.

In this study, demographic profiles, drug concentrations of PCP, and typical observed behaviors of subjects arrested for driving under the influence are presented where PCP was a significant toxicological finding from cases submitted to the Toxicology Laboratory of the Forensic Laboratory Division of the Office of the Chief Medical Examiner, City and County of San Francisco. Phencyclidine, PCP, was first developed in 1956 by Park Davis and investigated as a possible anesthetic. In clinical trials, some patients experienced a prolonged post-operative psychosis and it was withdrawn from clinical use in 1965. It is this adverse affect and the dissociative hallucinogenic properties of PCP which contributed to its popularity as a drug of abuse in the late 1960s. PCP use steadily declined over the next few decades, but recent data suggests there is resurgence in its use. The Drug Abuse Warning Network (DAWN) has presented data indicating that since 1999, there has been a general increase in PCP related visits to Emergency Departments.

All blood samples collected from impaired drivers are screened for ethanol and in those cases where ethanol concentrations are below 0.08 and drugs are suspected, a drugs abuse panel may be requested by the submitting agency. ELISA screening (Venture Labs, Inc.) was performed for amphetamine, barbiturates, benzodiazepines, cocaine, fentanyl, methadone, methamphetamine, opiates, oxycodone, phencyclidine, propoxyphene, and tricyclic antidepressants. Screened positives are confirmed by GC-MS.

In 2005, the SF-OCME's toxicology laboratory investigated 209 cases of suspected driving under the influence of drugs and 3 were positive for PCP (an incidence of 1.4%). In 2006 there were again 3 positive PCP drivers out of 183 submitted cases (an incidence of 1.6%) and in 2007 there were 6 PCP positive cases out of 170 submitted (an incidence of 3.5%). Reported here is the data from 13 PCP positive drivers, who were arrested for drug impaired driving. They were predominantly male (92%), had a mean and median age of 40, and in 62% PCP was the only psychoactive drug detected. The mean PCP concentration was 0.09 mg/L (range (0.03 – 0.20 mg/L). PCP positive drivers were significantly impaired with marked sedation, slurred speech, and when performed, subjects did poorly on field sobriety tests.

The incidence of driving under the influence of PCP in San Francisco is low but appears to be increasing (1.4% in 2005 to 3.5% in 2007), at the same time that the number of drivers for whom blood was submitted to the laboratory declined. The average age of PCP drivers is higher than the average age of other drug impaired drivers and higher than PCP impaired drivers reported from other jurisdictions. The drug concentrations appear to be higher in San Francisco than those reported in other studies. In conclusion, PCP continues to be found in San Francisco drivers arrested for DUID, thus making it important to continue screening suspected drug impaired drivers for PCP as they tend to be both severely impaired and have little or no ability to safely operate a motor vehicle.

PCP, Impaired Driving, San Francisco

K35 Sensitive Method For Detection of Cocaine and Metabolites by Liquid Chromatography Tandem Mass Spectroscopy (LC - MS/MS) in Urine

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After attending this presentation, attendees will be able to understand the uses of an assay for cocaine and metabolites with a lower limit of detection.

This presentation will impact the forensic science community by describing a method with a low limit of detection for cocaine and metabolites that will improve the ability to investigate drug use patterns.

Cocaine (COC) is an alkaloid found in *Erythroxylon coca*, and is a potent CNS stimulant that results in a state of increased alertness and euphoria. Cocaine can block the reuptake of neurotransmitters norepinephrine, dopamine, and serotonin. COC is rapidly metabolized to major metabolites like benzoylecgonine (BE) which is further metabolized to minor metabolites like m-hydroxybenzoyleconine (HOBE) and Norcocaine (NC). Cocaethylene (CE) is formed by trans-

esterification of cocaine with ethyl alcohol when used simultaneously. Anhydroecgonine methyl ester (AEME) is a unique metabolite of smoked cocaine, and anhydroecgonine ethyl ester (AEEE) has been identified in cocaine smokers who also use ethyl alcohol. A method has been developed for the detection of and quantiation of COC, HOBE, AEME, AEEE, NC, CE, and BE in urine at low concentrations.

Sixty-eight randomly selected previously confirmed BE positive by GC-Ms were collected from May 19th to 22nd and June 20th to 27th. Samples were extracted using SPE and 30uL of the reconstituted extract was injected. Chromatographic separation was achieved using a gradient consisting of Mobile phase A [20mM ammonium formate (pH = 2.7)] and Mobile phase B [50%/50% methanol/acetonitrile], and an XDB-C8, 1.8micron, 2.1x50mm column, with a flow rate of 270 ul/min. Concentrations were calculated by comparing the drug peak area with the internal standard peak-area. The ratio was plotted against a standard curve.

The assay displayed linearity from 1.0m- 1000 ng/mL for all analytes. Between-run CVs were <10% throughout the linear range. Of the 68 BE positive samples, 56 were positive for cocaine, 32 for AEME, 7 for AEEE, 64 for HOBE, 29 for NC, and 32 for CE at > 1.0 ng/mL.

Standard surveys to determine methods of cocaine use rely on individual responses to survey questions, and assumes that users responses are honest. We report here on a sensitive method to identify cocaine metabolites at concentrations as low as 1.0 ng/mL. Because of the unique derivation of some of the metabolites, the pattern of the metabolites makes it possible to determine the user's method of cocaine ingestion. This assay could be used to validate or challenge current survey techniques.

Cocaine, Cocaine Metabolites, LC-MS/MS

K36 Opiate Withdrawal and Adulterated CNS Depressant Drug Impaired Driving: Case Interpretation and Overcoming Motions to Suppress

Michael Wagner, PhD, State Department of Toxicology, Indiana University, 550 West Sixteenth Street, Indianapolis, IN 46202*

After attending this presentation, attendees will understand the key medical legal issues addressed when evaluating impaired driving resulting from opiate addiction. Case history, self reporting, biological samples, analytical test results, relevant case law, and strategies challenging motions to suppress will be discussed. In addition, the opposing conclusions from the expert witnesses will be presented.

This presentation will impact the forensic community by interpreting DUID cases involving impairment when only trace analytical test results are available.

On March 7th at 1445 hours, police are notified of a collision involving two vehicles (vehicle #1 a 1998 Nissan Frontier (pickup truck) and vehicle #2 a 1987 Volvo 740). The weather was clear and dry. Vehicle #2 was traveling eastbound and made a left hand turn (northerly) when it was struck broadside by vehicle #1 that was traveling westbound. Vehicle #1 driven by a 47-year-old male had violated a red traffic control device just prior to this collision. As a result of this accident, two males ages 17 and 34 occupying vehicle #2 had been fatally injured.

Scene evaluation of the driver for vehicle #1 reveals the subject claims to have been reaching down for a water bottle and taking his attention off the roadway. The subject made statements at the scene to police officers and medical personnel that he was a heroin addict and had ingested three Klonopin® prescription pills approximately 09:00 – 09:30. He crushed two of the pills and snorted them, and then he ingested the third. Subject claimed to have eaten very little in the past two days (two bowls of cereal the previous day) and nothing that day (body height and weight were 6'1" and 135 pounds). In addition, the subject claimed to be in withdrawal with his last use of Heroin having been on March 5th. The subject admitted to be in route to purchasing more Heroin and having $2,500 dollars in cash. Multiple blood samples were collected and indicated the presence of trace parent cocaine and metabolite, trace clonazepam, while urine test results confirmed the presence of morphine and oxycodone in addition to parent cocaine and benzoylecognine.

DUID, Opiate Impairment, Benzodiazepines

K37 Prescription Drugs, Poor Driving, DRE Evaluation…and a Surprising Verdict – A DUID Case Study

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After attending this presentation, attendees will have a greater understanding of drug interpretation, prosecutor preparation, and effective expert witness testimony for prescription drug impaired driving cases.

This presentation will impact the forensic community by influencing toxicologists who are involved with suspected DUID cases by enhancing their understanding of the challenges to interpretive issues.

For drugs other than alcohol, interpretation of drug concentrations and effects on safe driving ability is extremely complex. The toxicologist must consider drug pharmacology, pharmacokinetics, drug interactions, medical information, and research findings and apply them to the individual case scenario. This information must then be presented to the attorneys during preparation for the trial.

The case study that will be presented involves several drugs that can severely affect driving abilities. The drugs include oxycodone (at a potentially toxic concentration of 530 ng/mL), diazepam, nordiazepam, cyclobenzaprine and citalopram. Poor driving was observed by a citizen driver, reported to law enforcement, and documented by the arresting State Patrol Officer. A Drug Recognition Expert (DRE) was called to the scene and conducted an evaluation of the driver. The DRE concluded that he was impaired and under the influence of a CNS Depressant and Narcotic Analgesics.

At trial the driver alleged that he was able to ingest several oxycodone pills while in custody and prior to the blood sample collection. Even though this case had all the required elements for a DUID conviction, the first hearing resulted in a mistrial and was subsequently retried. The full case will be presented with emphasis given to drug interpretation, pharmacokinetics, prosecutor preparation, and effective expert witness testimony.

Drugs, Driving, Impairment

K38 Driving Under the Influence of Methamphetamine in the City & County of San Francisco, California

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After attending this presentation, attendees will have a better understanding of the signs and symptoms often observed in drivers driving under the influence of methamphetamine, the measured concentrations of the drug in the drivers' blood specimens, and the

incidence of methamphetamine in alleged DUID drivers in San Francisco, California.

This presentation will significantly impact the forensic community by providing reference epidemiological and toxicological data drawn from actual driving cases which can serve as tools in the investigation of alleged DUID cases involving methamphetamine in the United States and abroad.

In San Francisco, California, suspected DUID drivers are charged with violating Vehicle Code Section 23152(a) which relates to driving while a person's physical or mental faculties are impaired by alcohol (or drugs) to the extent that they are "unable to drive their car with the same caution characteristic of a sober person, of ordinary prudence, under the same or similar circumstances." A separate charge, 23152(b), relates to driving with BAC equal or greater to 0.08% (w/v). In this study we present the drivers' demographic profiles are presented together with the concentrations of methamphetamine, amphetamine, and related compounds in biological specimens submitted to the toxicology laboratory of the Forensic Laboratory Division of the SF OCME.

A computerized database (NIKTOX) and a manual search of reports were used to identify DUID cases in which methamphetamine and/or related compounds were detected and confirmed/quantified in biological specimens during a 3-year period (2005-2007).

In 2005, there were 209 cases of drivers suspected of driving in violation of 23152(a). Their age ranged from 17 to 82 years (median: 33 years). 171 of these drivers were male (82%). Methamphetamine was found in 17 cases and the age of those drivers ranged from 20 to 63 years (median: 37 years). 88% of the methamphetamine positive cases involved male drivers (n=15). Blood was collected in only 3 of the 17 cases. In the three blood cases, the methamphetamine and amphetamine concentrations were 0.6, 1.5, and 0.3 mg/L and 0.1, <0.1, and <0.1 mg/L, respectively.

In 2006, there were 183 cases of drivers suspected of driving in violation of 23152(a). This represented a decrease of 12% from the previous year. Their age ranged from 19 to 73 years (median: 33 years). Of these drivers, 157 were male (86%). Methamphetamine was found in 21 of the 183 cases. This represented an increase of 3.4% in methamphetamine incidence as compared to the previous year. The age of these 21 drivers ranged from 19 to 51 years (median: 28 years). Male drivers represented 71% of the methamphetamine positive cases (n=15) and 29% involved female drivers (n=6). The percentage of female drivers involved in methamphetamine DUID cases in 2006 represented more than a two-fold increase from the previous year. Blood was collected in 10 of the 21 cases and the median methamphetamine and amphetamine concentrations measured were 0.4 mg/L (range: <0.1 to 0.8 mg/L) and 0.1 mg/L (<0.1 to 0.1 mg/L), respectively.

In 2007, there were 170 cases of drivers suspected of driving in violation of 23152(a). This represented a further decrease of 7% in submissions as compared to the previous year. The drivers' age ranged from 17 to 82 years (median: 33 years). Of these drivers, 135 were male (79%). Twenty-five of the 170 cases were found to contain methamphetamine, a further increase of 3.2% in methamphetamine incidence from the previous year. The age of these 25 drivers ranged from 19 to 51 years (median: 33 years). Of these cases, 84% involved male drivers (n=21). Blood was collected in 12 of the 25 cases and the median methamphetamine and amphetamine concentrations measured were 0.3 mg/L (range: <0.05 to 0.7 mg/L) and 0.1 mg/L (range: <0.05 to 0.1 mg/L), respectively.

Methamphetamine incidence in driving under the influence cases almost doubled between 2005 and 2007 (from 8.1% to 14.7%) but in the same period the total number of DUID laboratory submissions by law enforcement agencies decreased by almost 19%. This suggests that driving under the influence of methamphetamine in San Francisco is on the rise but law enforcement agents in this jurisdiction may not be adequately resourced or adequately trained in the recognition and interception of drivers driving under the influence of substances other than ethanol. Additionally, women and younger drivers appear to be increasingly involved in methamphetamine DUID cases. It may be that greater efforts should be made in further educating our population of the risks associated with methamphetamine use and abuse instead of exclusively relying on the deterrent effects of fines and other penalties.

Methamphetamine, Driving, San Francisco

K39 Use of Serotonin Metabolites in Postmortem Alcohol Determinations

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After attending this presentation, attendees will more fully understand postmortem ethanol formation and ways to identify it.

Specimens from aviation accident victims are submitted to the FAA's Civil Aerospace Medical Institute (CAMI) for toxicological analysis. During toxicological evaluations, ethanol analysis is performed on each such case. Care must be taken when interpreting a positive ethanol result due to the potential for postmortem ethanol formation. Historically, ethanol distribution in various tissues and fluids from the same case and/or the presence of other volatile organic compounds at abnormal concentrations in these fluids and tissues has been employed as an indicator of postmortem microbial ethanol formation. However, these methods are not always reliable. The consumption of ethanol has been shown to alter the concentration of two major serotonin metabolites, 5-hydroxytryptophol (5-HTOL) and 5-hydroxyindole-3-acetic acid (5-HIAA). While the 5-HTOL/5-HIAA ratio is normally low, previous studies have demonstrated that the urinary 5-HTOL/5-HIAA ratio is significantly elevated following ethanol ingestion. The 5-HTOL/5-HIAA ratio is not affected by the microbial formation of ethanol, by consumption of serotonin-rich foods or by the use of SSRI's.

A single analytical approach has been developed to determine concentrations of both 5-HTOL and 5-HIAA that has provided a convenient, rapid and reliable solution to this problem. This novel methodology eliminates the need for two separate and unrelated analytical techniques, GC/MS and LC/EC, for the determination of these metabolites. The simultaneous determination of 5-HTOL and 5-HIAA in forensic urine specimens was achieved using a liquid/liquid extraction technique in conjunction with LC/MS. The ion trap MS used allowed us to perform MS/MS/MS on both 5-HTOL and 5-HIAA, and afforded limits of quantitation below 1 ng/mL for each compound. After development of this method, the previously established, antemortem, 15 pmol/nmol 5-HTOL/5-HIAA ratio cutoff was investigated and subsequently validated for use with forensic specimens.

The FAA laboratory utilizes this method to examine all postmortem ethanol-positive urines, where the source of ethanol is unclear. This presentation will discuss the difficulties in determining the source of ethanol in postmortem cases, markers of ethanol ingestion, and the application of this novel methodology in elucidating ethanol origin. Multiple case studies that involved postmortem alcohol formation will be presented.

Postmortem Ethanol, LC/MS, Serotonin Metabolites

K40 Clinical and Forensic Toxicology of

Gamma - Hydroxybutyrate Closely Resembles That of Ethanol

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After attending this presentation, attendees learn about the clinical and forensic toxicology of two widely used recreational drugs, namely the legal drug ethanol and the illicit drug gamma-hydroxybutyrate (GHB). Both substances are highly soluble in water have low molecular weight and their pharmacological effects are similar to the major central nervous system depressants (general anesthetic gases, barbiturates and benzodiazepines).

This presentation will impact the forensic community by teaching the similarities and differences in clinical pharmacokinetics of ethanol and GHB, the analysis and stability of these substances in blood during storage, the distribution between serum and whole blood, and the effects of food and gender on concentration-time profiles. Moreover, the toxicity of ethanol and GHB are compared and contrasted based on the concentrations determined in blood from impaired drivers and medical examiner cases.

Ethanol and GHB are produced naturally in the body and are measurable in blood and urine at very low concentrations of ~1 mg/L. For recreational purposes both drugs are taken orally and are rapidly absorbed from the gut and distributed into the total body water (TBW) compartment. The distribution of ethanol and GHB between the plasma and erythrocyte fractions of whole blood is similar to that of water distribution, suggesting serum/whole blood ratios of 1.15:1 (range 1.10 to 1.20). Ethanol and GHB don't bind to plasma proteins and undergo extensive hepatic metabolism with only a small fraction (2-5%) of the dose being recoverable in the urine. The metabolism of ethanol and GHB occur by capacity limited kinetics and mathematically this can best be described by the Michaelis-Menten equation. Human dosing studies have shown that when the concentrations in blood pass 150 mg/L (ethanol) and 10 mg/L (GHB), the metabolizing enzymes are virtually saturated with substrate and zero-order kinetics applies. After moderate doses, the elimination rate of ethanol from blood is within the range 100-200 mg/L/h compared with 10-20 mg/L/h for GHB. The terminal half-lives of ethanol and GHB are relatively short; being in the range 15-30 min. The apparent volumes of distribution (V_d) of both substances are 0.5-0.7 L/kg as expected for water-soluble, non-protein bound drugs that distribute into the TBW. Concentration-time profiles of ethanol and GHB after moderate doses were similar for men and women in terms of C_{max}, t_{max} and area under the curve (AUC). The rate and extent of absorption is slowed considerably if ethanol or GHB are ingested together with or after a meal, owing to delayed gastric emptying and first-pass metabolism. Under these conditions, C_{max}, t_{max} and AUC are markedly diminished compared with the same dose of the drugs taken on an empty stomach.

Both ethanol and GHB can be determined in blood and urine by conventional gas-liquid chromatography with a flame ionization detector, either by direct injection or headspace technique. Methods are also available for analysis of these substances by GC-MS, which permits use of deuterium labeled analogues as internal standards for unequivocal identification. The concentrations of ethanol and GHB in specimens of whole blood from impaired drivers were remarkably stable during storage at 4°C for several months after sampling.

The mean and median blood-ethanol concentrations in impaired drivers were 1,700 mg/L (N = 29,000) and in some instances the concentrations exceeded 4000 mg/L. These results can be compared with mean and median GHB concentrations of 89 and 82 mg/L (N = 548) in impaired drivers, highest 340 mg/L. The concentrations of ethanol and GHB in blood from living subjects overlapped with concentrations seen in drug-related deaths. The mean and median blood-ethanol concentration (N = 800) was 3600 mg/L and 3500 mg/L, respectively compared with mean and median GHB (N = 37) of 294 mg/L and 190 mg/L, respectively.

Capacity limited pharmacokinetics of ethanol and GHB needs to be carefully considered when the concentrations in blood after toxic doses are interpreted. The terminal half-life should not be used to make predictions about times necessary to eliminate ethanol or GHB from blood or the amount ingested after large recreational or abuse doses are taken. Interpreting the concentration of ethanol and GHB in medical examiner in terms of toxicity and whether drug intoxication was a possible cause of death is complicated by concomitant use of other psychoactive substances.

Ethanol, GHB, Toxicology

K41 Determining Concentrations of Fentanyl in Decomposing and (Formalin-Stored) Postmortem Liver Tissue Over Time by Gas Chromatography-Mass Spectrometry (GC-MS)

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After attending this presentation, attendees will have an overview of the opioid analgesic drug fentanyl, its stability over time in aqueous and liver matrices, and the effects of simulated embalming in formalin on its concentration.

This presentation will impact the forensic community by aiding the forensic medical examiner and forensic scientist in the determination of fentanyl concentrations in decomposing tissue cases as well as cases where tissue has been stored in formalin.

A systematic study of matrix effects on the postmortem concentration of the opioid analgesic drug fentanyl in liver tissue was conducted over a six-year period. Porcine liver homogenates were spiked with 200 nanograms of fentanyl per gram of liver to simulate a fatal overdose and treated with the chemical preservative formalin to simulate embalming of the deceased victim. The samples were prepared in triplicate (samples 1A-1C) and stored at room temperature. Periodically, aliquots were removed from the sample containers and extracted using a solid-phase extraction (SPE) method, and the concentration of fentanyl was monitored over time by gas chromatography-mass spectrometry (GC-MS). To isolate the effects of formalin and of the liver tissue itself on fentanyl's concentration, triplicate samples were also prepared in which these two components were systematically omitted from the sample sets (samples 2A-4C). Also, negative controls were prepared in which no fentanyl was spiked into the samples (samples 5A-6C). Statistical analysis of the concentration data over time was conducted to determine effects of time and other sample matrix components on fentanyl concentrations. Details of the study, data analysis, and results as well as implications for forensic toxicology practice will be presented.

Fentanyl, GC-MS, Solid-Phase Extraction, Formalin

K42 Homicide by Propofol

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After attending this presentation, attendees will understand how medications such as propofol can be used to murder individuals, and the investigative techniques available to identify such deaths.

This presentation will impact the forensic science community by describing a unique and difficult to investigate method of homicide.

In November 2005, a 24-year-old previously healthy woman was found dead in her residence in Gainesville, Florida after her boyfriend had been unable to reach her. She was found prone, facedown in the bed, and was fully clothed. No drugs or drug paraphernalia were found in the residence, and there were no signs of a struggle or other interpersonal violence. At autopsy, the body had fixed lividity on the ventral surfaces, with blanched areas on the forehead, nose, chin, and across the chest corresponding to the left arm under the body. A single, minute pinpoint puncture wound was identified on the left antecubital fossa, directly overlying a prominent subcutaneous vein in the antecubital fossa. Minimal hemorrhage was present in the intervening soft tissues. No other abnormalities were observed during the autopsy. Blood, urine, vitreous humor and tissue specimens were obtained and submitted for toxicological and histological studies.

Law enforcement personnel in attendance at the autopsy alerted those at the scene regarding the puncture wound, and subsequently, the investigation widened to include inspection of garbage containers outside of the residence. Investigators found vials of propofol, etomidate, midazolam, and saline, along with needles and intravenous prep materials.

The medications and medical paraphernalia were traced back to a local hospital and linked to a male acquaintance of the victim. The male acquaintance, who apparently was infatuated with the young woman, was an ICU nurse who coincidentally was terminated from his position shortly after the young woman's body was found. Although he was suspected to have involvement in this victim's death, several months passed before DNA evidence definitively linked him to the crime. During this intervening time, he left the region, and subsequently fled the country for Ireland.

Postmortem blood and urine specimens were subjected to comprehensive drug analysis including volatiles and over-the-counter, prescription and illicit drugs. The blood was positive for propofol (4.3 mg/L), phentermine (0.64 mg/L), and diphenhydramine (trace). In addition, 15 mg/L of GHB was detected in the urine.

Propofol is an intravenous anesthetic agent with rapid-onset of action and is primarily used for the induction and maintenance of anesthesia in surgical procedures, as well as a sedative in various clinical settings. Blood concentrations of propofol at approximately 4 mg/L are typically achieved for maintenance during major surgery, and individuals at these concentrations require mechanical ventilation.

With these results, in addition to the absence of significant anatomic findings, the death was certified as propofol intoxication, and the manner of death was certified as homicide. Pursuant to the conclusion of the medicolegal death investigation, a warrant was issued for the male acquaintance's arrest. Eventually he was captured in Senegal, extradited to the U.S., and formally charged and tried for the death of the young woman. The male acquaintance was found guilty of first-degree murder and sentenced to life without parole.

Homicide, Propofol, Death Investigation

K43 Five Fatal Occupational Injuries Due to Gas Inhalation Occurred During Truck-Tank Washing Operation: Environmental Findings

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After attending this presentation, attendees will be briefed on five cases of fatal asphyxiation at work, which occurred during a truck-tank washing operation.

This presentation will impact the forensic community and/or humanity by demonstrating how fatal deaths were caused by gaseous hydrogen sulfide (H_2S), a byproduct of the chemical compound being shipped, and that the accumulation of toxic gases in a closed space can induce asphyxiation in a matter of minutes.

During a routine truck-tank washing operation, a worker got into the empty tank through the upper pothole and in a few minutes fell unconscious. Assuming an accident happened, a second worker went into the tank where he also fell unconscious. The last three men died trying to remove their co-workers out of the tank. Before the washing operation, the tank had previously contained sulfur liquid. All five workers had been in good health and had had a mean age of 37.6 years (range 20-64).

To clarify the cause of death and identify the factors involved in asphyxia, it is crucial to identify the fatal compound(s) and its/their origin. Therefore, several specimens from the tank were characterized and a simulation on two analogous truck-tanks was also carried out.

Dregs of a blackish liquid and a yellowish granular solid from the tank bottom were analyzed using headspace/GC-MS technique. Air samples were analyzed using commercial available color dosimeter tubes and H_2S quantitative determination was also performed in liquid sulfur. Thiosulfate, was measured in blood samples by GC/MS technique after derivatization with pentafluorobenzyl bromide.

Analyses confirmed that the dregs of yellowish solid samples were composed of sulfide. The blackish liquid was a mixture mainly consisting of liquid sulfide and H_2S as contaminant (2.5 mg/l). The absence of hydrocarbon-aliphatic compounds and solvents together with its almost neutral pH (7.6) demonstrated that the workers had not used detergents or basic compounds.

Air monitoring at the third opening inside the tank (one week after the accident), revealed high H_2S concentration (> 60 ppm) while sulfur oxides were negligible, which excluded a sulfur combustion induced by the workers. At the fourth opening (one month after the accident), H_2S air concentration was less than 0.25 ppm. This depletion was due to the continuous opening of the porthole during the rescue operation and the following inspections, as demonstrated by the strong characteristic odor of rotten egg that could be smelled in the area outside the truck-tank. The high H_2S concentration in the air inside the tank was ascribed to the contamination of the original liquid sulfur, produced by Claus's process, the most significant industrial process used to recover elemental sulfur from gaseous hydrogen sulfide.

To support the hypothesis that H_2S rising from liquid sulfur was responsible for the deaths, two similar truck-tanks used for liquid sulfur transport, and the sulfur itself were also tested. Before loading liquid sulfur, air inside the tanks contained only O_2 (20.9 % v/v). During the

loading phase H_2S air concentrations were 41 and 71 ppm, respectively and became 80.9 and 600 ppm when the tanks were fully loaded.

The liquid sulfur analyzed revealed high contaminations of H_2S: 85 and 108 mg/Kg.

According to Henry's Law (K_H=0.087 Pa•m^3/mol), H_2S tends to pass towards the gas phase. This evaporation is favored by the movement of the liquid (e.g. during the shipping) that increases the kinetics of evaporation. Therefore, after liquid sulfur had been removed from the tank bottom, H_2S remained in the gas phase causing the asphyxiation of the workers, as confirmed by the pathological and toxicological findings. In fact, abnormal concentrations of thiosulfate, the major metabolite of H_2S, from 0.023 to 1.63 mmol/l (average value: 0.38 mmol/l) were revealed in all *postmortem* blood samples.

Environmental and biological results confirmed that H_2S fumes were responsible for the multiple deaths and no other adverse reactions that happened inside the tank. This report presents valuable findings in correctly identifying the cause of death in gas asphyxiation cases.

Asphyxia, Hydrogen Sulfide, Occupational Accident

K44 Nine Xylazine Related Deaths in Puerto Rico

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After attending this presentation, attendees will understand the nature of lethal risks posed by xylazine and will be familiarized with toxicological and pathological findings of nine xylazine related deaths. The presentation will raise awareness among the forensic community, law enforcement, clinicians and the general public regarding the potential lethality of xylazine used alone or in combination with other drugs.

This presentation will impact the forensic community by providing a better understanding regarding the lethal risks of xylazine and increasing awareness of the presence of this substance as an adulterant of illicit drugs and its role as a cause of death.

Xylazine is a phenilaminothiazine derivative, structurally related to clonidine that is employed as a veterinary sedative, analgesic, and anesthetic that has been proven harmful to humans. In the mid 1960's xylazine was investigated as a sedative hypnotic/analgesic premedication in humans, but was rejected because of its frequent association with severe hypotension. In humans, toxicity consists of central nervous system depression, bradicardia, and hypotension. Its pharmaceutical action results in sympathetic discharge via stimulation of alpha-2-adrenoreceptors.

Xylazine has been frequently found as an adulterant of illicit drugs, mainly heroin. Both drugs are dangerous to humans, and due to their similar pharmacologic effects, drug synergy can occur.

Researchers reviewed nine cases occurring within the period 2003-2007 at the Puerto Rico Institute of Forensic Sciences (PRIFS) in which xylazine was detected and determined to be the cause of death. Xylazine was detected and quantified in blood using Liquid Chromatography/Mass Spectrometry (LC/MS).

The nine cases of xylazine related deaths are summarized in Table 1. In eight of the nine cases, the individuals were found unresponsive and pronounced dead at the scene. The scene was the decedent's residence in five of the eight cases, in two cases it was the street, and in one case a hospital room. Case #9 complained of shortness of breath, had a witnessed collapse at his residence, and died minutes later. History of drug abuse was present in all cases. Five were males and four were females whose ages ranged from 23 to 70 years. At autopsy there was no external or internal trauma in any of the cases. Recent venipuncture sites in the upper extremities were found in two cases. Internal examination was remarkable for moderate to severe pulmonary congestion and edema, a common finding for all cases. No additional pertinent autopsy findings were noted. Toxicological analyses disclosed the presence of blood xylazine levels (range 0.29 – 5 µg/mL) and morphine (range 0.08 – >1 µg/mL) in all cases. Cocaine was detected in three cases and ethanol in four cases. The cause of death was determined to be toxic effects of xylazine and opioids for all cases. Additionally cocaine and alcohol were included in cases in which they were detected. The manner of death was accidental in all nine cases.

According to a recent study of the street heroin samples analyzed by the Control Substance Laboratory of the PRIFS, xylazine was found in 36% of the cases as a heroin adulterant. Given the potential toxicity and lethality of xylazine when used alone or in combination with heroin or other drugs, it is necessary to be aware of the emergence of this substance in the community and consider methods of improving its detection. There are limited reports of human toxicity and deaths related to xylazine. The toxicological and pathological aspects of nine cases are reported and discussed with all of the literature available to date.

Table 1: Nine PRIFS Xylazine Related Deaths

Cases	Gender	Age	Drugs	Blood (µg/mL)
1	M	29	Xylazine	0.29
			Morphine	0.17
			Cocaine	0.13
			Benzoylecgonine	0.66
2	F	35	Xylazine	0.50
			Morphine	0.21
			Benzoylecgonine	0.13
			Ethanol	0.20%
3	M	23	Xylazine	0.70
			Morphine	0.07
4	F	45	Xylazine	0.70
			Morphine	> 1.00
			6-Monoacetylmorphine	0.03
			Codeine	0.08
5	M	53	Xylazine	0.30
			Morphine	0.26
			6-Monoacetylmorphine	0.01
			Cocaine	0.10
			Benzoylecgonine	> 1.00
			Ethanol	0.14%
6	F	70	Xylazine	1.90
			Morphine	0.08
			Cocaine	0.19
			Benzoylecgonine	0.43
7	M	49	Xylazine	5.00
			Morphine	0.27
			6-Monoacetylmorphine	0.01
			Ethanol	0.12%
8	F	30	Xylazine	2.00
			Morphine	0.43
			6-monoacetylmorphine	0.02
			Benzoylecgonine	0.11
			Ethanol	0.36%
9	M	30	Xylazine	0.41
			Morphine	0.13
			6-Monoacetylmorphine	0.02
			Benzoylecgonine	0.10

Xylazine, Heroin, Cause of Death

K45 Common Heroin Adulterants in Puerto Rico: The Emergence of Xylazine

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After attending this presentation, attendees will learn about common adulterants of heroin and the emergence of xylazine as the main adulterant of heroin in Puerto Rico. The goal of this study is to increase the awareness about the appearance of these drug combinations and their potential toxic effects.

This presentation will impact the forensic community by presenting statistical information about the use of xylazine, a drug that was identified as the most frequent adulterant of the street heroin in Puerto Rico.

Xylazine is marketed as a veterinary drug and used as a sedative, analgesic, and muscle relaxant for large animals, such as deer, ruminants, and horses. Xylazine is not approved for human use because it has been proven harmful to humans. Only 27 cases of toxicity caused by xylazine consumption have been documented in humans. According to these reported cases, consumption was accidental, suicidal or for homicidal purposes, occasionally resulting in death. Xylazine was detected and reported as the cause of death in nine postmortem cases from the Puerto Rico Institute of Forensic Sciences (PRIFS).

Illicit drugs such as heroin are often adulterated (cut) with other substances to either enhance or diminish the drug effects and to increase the weight and volume of the drug, thus increasing the dealer's profits. Many different substances are used to cut heroin. Some of the more common non-opiate cutting agents with pharmaceutical effect encountered by the Controlled Substances Section of PRIFS were: caffeine, procaine, cocaine, quinine, lidocaine, and the most frequently detected substance, xylazine. In 2007, a total of 663 suspected street heroin items (or exhibits) were analyzed qualitatively by gas chromatography/mass spectrometry (GC/MS). Heroin was present in 92% (613) of the total items. Of the remaining 8% (50) of the items, 40 (80%) items had xylazine as the main drug. These 40 items represent 6% of the total 663 analyzed items (Table 1).

From the 613 positive heroin items, heroin was identified in 43% (265) as the only drug. In 57% (348) of the items heroin was found in combination with other drugs. The most common heroin combinations were heroin/xylazine (36%), heroin/caffeine (22%), heroin/xylazine/caffeine (13%), heroin/quinine (9%), heroin/cocaine (4%), heroin/xylazine/cocaine (3%), heroin/xylazine/quinine (3%) and other drugs combinations (Table 2). Of the 348 heroin items, 199 (57%) had xylazine as an adulterant. Figure 1 shows a typical chromatogram result obtained from a street sample folded in a sheet of aluminum foil (Figure 2).

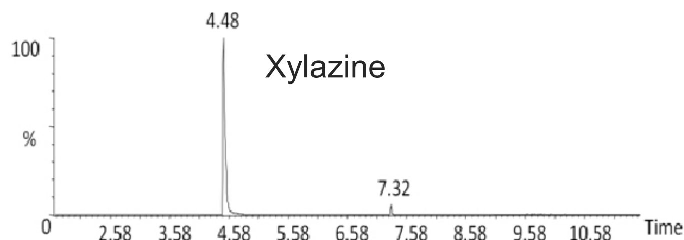

Figure 1. Chromatogram showing a typical street heroin item received by the Controlled Substances Section of PRIFS.

Table 1. Number and percentage of items without heroin, 2007 PRIFS

Table 1. Number and percentage of items without heroin, 2007 PRIFS	Total	Percentage
Xylazine	32	64
Xylazine/Caffeine	1	2
Xylazine/Caffeine/Cocaine	1	2
Xylazine/Caffeine/Cocaine/Lidocaine	1	2
Xylazine/Cocaine	1	2
Xylazine/Quinine	4	8
Caffeine	3	6
Caffeine/Lidocaine	1	2
Caffeine/Quinine	1	2
Quinine	5	10
Total	50	100

Table 2. Most Frequently Identified Heroin Combinations Number and percentage of identified heroin combinations, 2007 PRIFS

Table 2. Most Frequently Identified Heroin Combinations Number and percentage of identified heroin combinations, 2007 PRIFS	Total	Percentage
Heroin/Xylazine	125	36
Heroin/Xylazine/Caffeine	45	13
Heroin/Xylazine/Cocaine	9	3
Heroin/Xylazine/Quinine	9	3
Heroin/Xylazine/Lidocaine/Procaine	3	1
Heroin/Xylazine/Caffeine/Lidocaine	2	1
Heroin/Xylazine/Caffeine/Procaine	2	1
Heroin/Xylazine/Other combinations	4	1
Heroin/Caffeine	75	22
Heroin/Caffeine/Quinine	5	1
Herion/Caffeine/Lidocaine	3	1
Heroin/Quinine	33	9
Heroin/Cocaine	13	4
Heroin/Cocaine/Other combinations	4	1
Heroin/Lidocaine	4	1
Heroin/Procaine	4	1

The results of this statistical information show that xylazine is the main adulterant of the street heroin in Puerto Rico. Xylazine not only was found as an adulterant of heroin but also was found as the only component or in combination with other drugs. Xylazine may be fatal when used in combination with heroin or with other drugs. The combination of heroin and xylazine can elicit synergistic effects. Literature shows some similar pharmacologic effects between xylazine and heroin. Further studies are suggested to increase the knowledge and understanding of this emerging drug as an adulterant of heroin.

Xylazine, Heroin, Adulterants

K46 Validation of a Method for the Determination of Opiates and Methadone in Hair

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After attending this presentation, attendees will have an insight into the methodology used to develop and validate a forensic toxicology method for hair analysis of opiates and methadone to IEC/ISO 17025:2005 standards within an accredited laboratory.

This presentation will impact the forensic community by providing laboratories considering obtaining accreditation with an insight into the methodology required for method validation.

The purpose of this study was to develop and validate a procedure for the determination of morphine, 6-acetylmorphine, codeine, dihydrocodeine, methadone, and EDDP in hair. Deuterated internal standard mixture and 0.1M HCl were added to 20 mg of specimen, control or spiked blank hair and sonicated for 1 h. The analytes were then extracted by solid-phase and derivatized with BSTFA + 1% TMS prior to GC-MS-SIM analysis. The limits of quantitation were <100 pg/mg for all drugs and the limits of detection <50 pg/mg. The intra-day and inter-day precisions of the assay were determined at 500 ng/mg and 2000 ng/mg and were <10% for all drugs.

An evaluation of the suitability of internal and external control samples was carried out throughout the validation process. Internal and external controls consisted of either spiked blank hair samples or pooled positive hair samples. The validation process found the controls to be effective and laboratory methodology was amended for their inclusion in all subsequent batch analyses. The validation data demonstrate that the method for the analysis of opiates and methadone in hair is sufficiently reproducible, robust and sensitive to carry out routine analysis within an IEC/ISO 17025 accredited laboratory.

Hair Analysis, Opioids, ISO 17025

K47 Postmortem Analysis of Cocaine, Benzodiazepines, Opiates, and SSRIs in Hair

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The goal of this study is to compare the occurrence of drugs in hair with findings in corresponding femoral blood specimens from several medicolegal death investigations.

This presentation will impact the forensic community by explaining how hair specimens can provide a unique avenue of study in cases of severe decomposition, embalming, or chronic fetal drug exposure.

The purpose of this study was to compare the occurrence of drugs in hair with findings in corresponding femoral blood specimens from several medicolegal death investigations. Hair specimens can provide a unique avenue of study in cases of severe decomposition, embalming, or chronic fetal drug exposure. Extracts from hair can be applied to ELISA, LC-MS, and GC-MS analytical techniques for drug screening and confirmation. In the present study, hair extracts were evaluated by ELISAs according to the shown cut-off values, and also assessed by LC-MS/MS library matching. These results were compared to the drugs found in the corresponding femoral blood collected at autopsy.

ELISA cut-off values (pg/mg)

Carisoprodol	1000
Cocaine	500
Benzos	200
Fentanyl	20
Methadone	200
Opiates	200
Oxycodone	300
Tramadol	1000
Propoxyphene	200
Amphetamine	500

Parent drugs, as well as some of the metabolites, accumulate within the hair cortex as the follicle grows. This evidence of drug use is stably incorporated into the hair, and does not usually diminish with standard hygienic practices. It is possible for drugs to associate with hair indirectly through sweat and sebum secretions, as well as contact with drug powder or smoke. However, when proper external decontamination is applied in combination with the presence of biologically derived drug metabolites, issues surrounding lingering external contamination diminish.

Hair must be broken down to release the drugs trapped within the protein structures, which can often destroy drug evidence or obscure the interpretation of parent/metabolite ratios. Current methods involve the use of mechanical or harsh chemical treatments to degrade hair. Mechanical disintegration requires specialized equipment, and strongly acidic or alkaline chemical treatments can further degrade the compounds of interest. This work describes methods for analyzing the drugs in hair with minimal effort, without destroying evidence.

Ten postmortem hair specimens were chosen for evaluation, which represent a wide variety of putative cocaine, benzodiazepine, SSRI, opiate, fentanyl, and methadone combinations based on prescribed and historical drug use. The hair samples (10-20 mg) were washed, dried, and weighed before further analysis to remove external contaminants. Extracts for ELISA analysis were prepared by incubating hair specimens in phosphate buffer for 2 hours at 60ºC. These formulations were diluted 1:5 in phosphate buffer before analysis. For LC-MS/MS analysis, the specimens were immersed in a 200 mM dithiothreitol solution, supplemented with a 100 ng/mL mepivacaine internal standard. Following 2 hours incubation, the extract was diluted with a 0.2% formic acid/20% acetonitrile solution, then filtered and stored in autosampler injection vials. The yield of drugs produced by this method was sufficient to apply towards LC-MS/MS screening library and confirmation assays.

The results obtained from these methods correlated well with drugs found in femoral blood extracts, where the cause of death was usually attributed to combined drug toxicities. The presence of other drugs in hair that were not present at the time of death illustrated a history of use. Additionally, the presence of other alkaloids and adulterants were found, such as noscapine and lidocaine, which supports the evidence of illicit sources of opiates and cocaine. Such contaminants are usually found in clandestine compositions, while pharmaceutical companies provide cleaner preparations. These detection procedures are technically feasible and efficient methods for releasing drugs trapped within hair for the purposes of forensic toxicology analysis, which aids in describing a pharmacological history of the decedent.

Postmortem, Hair, Toxicology

K48 Evaluation of Alcohol Markers in Postmortem Hair and Blood: Comparison Between Ethyl Glucuronide, Ethylsulphate, and CDT

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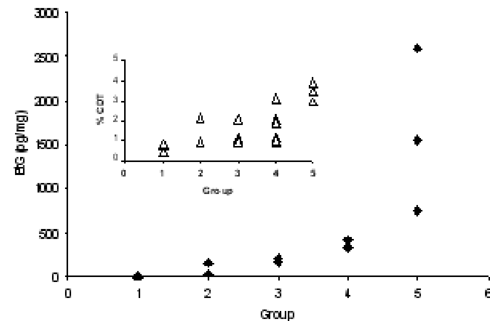
After attending this presentation, attendees will understand the incorporation of EtG and EtS in hair as well as the use of CDT measurements to diagnose chronic alcohol use in the deceased.

This presentation will impact the forensic community by presenting new analytical technique and data on new markers of alcohol abuse.

Forensic medicine primarily deals with investigation of apparent or suspected unnatural deaths. Analysis of alcohol and the interpretations of its influence may be crucial in the investigation of traffic accidents, suicides, and homicides, but also in other cases. Since chronic alcoholism is one of several underlying diagnosis that can explain the cause of death in obscure cases, identification of heavy alcohol abuse is an important issue in forensic medicine. However, markers of alcohol over-consumption have previously been criticized for not having sufficient specificity. As a result of this, identification of alternative markers has been encouraged. Measurement of CDT and phosphatidyl ethanol has previously been evaluated in postmortem population and recently there has been interest in direct markers of ethanol consumption. Ethyl glucuronide (EtG) and ethyl sulphate (EtS) are exclusively formed after ethanol exposure, and is incorporated in hair. This study was performed to provide diagnostic improvement of alcohol abuse in forensic medicine by comparing the findings of EtG and EtS in hair with that of blood CDT as well as with the medical history of the deceased.

The study was approved by the Regional Research Ethics Committee in Linköping (#M47-08). The study material was collected at the Departments of Forensic Medicine in Stockholm, Linköping and Lund. Forensic nurses interviewed the relatives of deceased persons and retrieved information from medical journals and police reports to investigate the alcohol history of the persons. From each subject, samples of hair and blood were collected and analyzed for EtG, EtS and CDT. Based on the background information, the subjects were divided into five groups: persons with no or limited alcohol intake (N=5), occasional drinkers (N=4), moderate drinkers (N=8), alcohol abusers (N=11), and excessive alcohol abusers (N=15). EtG and EtS in hair were measured by ultra performance liquid chromatography/electrospray tandem mass spectrometry (UPLC/ESI-MS/MS) on a 3 cm portion of the hair. These results were compared with reported alcohol consumption, and with blood levels of CDT determined by HPLC (see Table for mean values). In total, 43 deceased subjects were included in the study. A correlation was found between EtG and EtS levels in hair. EtG correlation with background information was probably blurred by uncertain background information, but when only cases with a high degree of reliability concerning alcohol intake information were included differences between the groups became more pronounced (See Figure). Only 19 of the blood samples could be analyzed for CDT owing to problems obtaining "postmortem" serum resulting in matrix interferences in the HPLC chromatogram. In eight of the cases CDT levels above 2% indicated overconsumption (see Figure insert). A low correlation (R^2= 0.28) between EtG in hair and CDT in serum was found. One explanation for this might be that the time windows are different, CDT being elevated 4-6 weeks after cessation of drinking whereas EtG in hair had longer detection time because of the 3-cm hair length analyzed. Using a 30 pg/mg cut-off, all but three cases should have been diagnosed as over consumption, including two of the cases with limited consumption reported. In conclusion, EtG and EtS showed similar trends and no preference for the other could be discerned. CDT was difficult to analyze in more than half of the samples. Further studies including a larger number of study objects and more reliable background information are required before a final cut-off value can be established.

Group	1	2	3	4	5
EtG (mean pg/mg)	361	398	493	541	907
EtS (mean pg/mg)	220	618	1054	975	2604
CDT (mean %)	0.7	1.5	1.3	1.7	3.3



Ethyl Glucuronide, Ethyl Sulphate, CDT

K49 Forensic Toxicology Findings in Blood and Urine From Female Victims of Alleged Sexual Assault

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After attending this presentation, attendees will acquire up-to-date information about the occurrence of ethanol and other drugs in blood and urine from female victims of alleged sexual assault in Sweden.

This presentation will impact the forensic community by providing data on the most common drugs found in female victims of alleged sexual assault in Sweden and will also aid forensic toxicologists in the interpretation of such cases.

Cases of alleged drug facilitated sexual assault (DFSA) have been increasingly reported in forensic science and medical journals since the 1980s. In news media the term "date rape" is often seen to describe such cases, although toxicologists and medical practitioners prefer the acronym DFSA, which implies use of a chemical agent to facilitate non-consensual sexual contact. The prevalence and the types of drugs encountered during investigations of alleged sexual assault are likely to differ between countries depending on social norms and the availability and popularity of recreational drugs. Ethanol, either alone or together with other drugs, has been a common finding in many previous surveys of DFSA from the United States and United Kingdom. Indeed, some victims suspect that their drinks had been spiked with a drug to explain the condition they found themselves in e.g., sudden incapacitation, sedation and subsequent drug-induced amnesia. Fast acting sedative-hypnotics such as gamma-hydroxybutyrate (GHB) and flunitrazepam have frequently been associated with so called "drink spiking". However, compelling evidence that a person was incapacitated by voluntary or involuntary consumption of alcohol and/or drugs is not easy to obtain from results of toxicological analysis.

The population of Sweden is just over 9 million and forensic toxicology is done at one central laboratory. The results of toxicological analyses for ethanol and drugs are entered into a database (ToxBase)

along with age and gender of the individuals concerned. All cases registered between 2003 and 2007 where indications existed that a female had been subjected to some form of sexual assault were included in this study (N = 1,806). In many cases the police made a special request for toxicological analysis for certain substances because intoxication and "date rape" already formed a part of the investigation. During examination of the victim by a physician, which often occurred several hours after the event, specimens of blood and if possible urine were taken and sent for toxicological analysis. This entailed a broad screening by use of immunoassay methods (EMIT/CEDIA) on urine if available or on blood after protein precipitation. All positive results from screening were verified by more specific methods, involving isotope dilution gas chromatography-mass spectrometry. The analysis of a large number of prescription drugs was done by capillary column gas chromatography with a nitrogen-phosphorous detector. The concentrations of ethanol in blood and urine were determined by headspace gas chromatography and values below 0.1 g/L were reported as negative.

The present series of 1,806 cases consisted of blood only in 207 cases (11.5%), both blood and urine in 1,431 cases (78.7%), only urine in 170 cases (9.4%), and hair in 8 cases (0.4%). Accordingly, blood samples were available for toxicological analysis in 90% (N = 1,628) of cases of alleged sexual assault over the 5-year period. The number of cases of DFSA was highest during the warmer summer months (June-September) and the mean age of victims was 24 y (median 20 y), with ~60% being between 15 and 25 years. In 559 cases (31%) ethanol and drugs were negative, 772 cases (43% of total) contained ethanol alone, 215 cases (12%) contained ethanol with at least one other drug and 262 cases (15%) contained either licit (prescription) or illicit drugs (amphetamine, ecstasy, cannabis, cocaine, GHB). The mean, median and highest blood concentrations of ethanol (N = 806) were 1.24 g/L, 1.19 g/L and 3.7 g/L, respectively. Higher blood-ethanol was associated with increasing age of victims ($r = 0.365$, $p<0.001$). Amphetamine (N = 86) and tetrahydrocannabinol (N = 100) were the most common illicit drugs identified in blood at mean (median) concentrations of 0.22 (0.10) mg/L and 0.0012 (0.0006) mg/L, respectively. A wide spectrum of prescription drugs was identified in these cases, including sedatives such as diazepam (N = 88), alprazolam (N = 55) and zopiclone (N = 35), but mostly at therapeutic concentrations. Among non-sedatives, antidepressants (SSRIs), paracetamol and opiates (codeine and tramadol) were the major findings. These drugs might have been used to treat a legitimate medical condition and have nothing to do with chemical submission or DFSA. However, finding high therapeutic concentrations of drugs with short half-lives, such as zolpidem and zopiclone, makes it more likely that sedation and DFSA was involved.

Ethanol was by far the dominant psychoactive substance identified in blood and urine samples in these DFSA cases and this agree well with studies from other countries (e.g. USA and UK). T he high average blood-ethanol concentration of 1.24 g/L at time of sampling verifies that considerable amounts of alcohol had been consumed, especially if a back extrapolation of the concentration to time of attack is made. Relating the blood-concentrations of ethanol and/or drugs to the degree of incapacitation and helplessness of the victim is fraught with difficulties.

Drugs, Ethanol, Sexual Assault

K50 Validation of a Color Test for Gamma-Hydroxybutyrate and Gamma-Butyrolactone

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After attending this presentation, attendees will have a better understanding of the ferric hydroxamate color test for the detection of gamma-hydroxybutyrate (GHB) and gamma-butyrolactone (GBL).

This presentation will impact the forensic science community by serving to provide a validated effective screening/presumptive color test for the detection of GHB in evidential samples.

GHB is one of the most widely-used drugs for drink adulteration in "date-rape" cases. Several test kits have been introduced to detect GHB in drinks; however, presently there is no single effective color test available for use by forensic drug laboratories to screen evidential submissions for GHB and GBL. Previously, Michalik et al.[1] described the creation of a new colorimetric spot reagent for the detection of GHB and GBL. This colorimetric spot reagent was an adaptation of the ferric-hydroxamate test for lactones.[2] It is a simple test that requires little sample preparation, and takes just a few seconds to accomplish. The test is able to detect 1 mg/mL of GHB or GBL in solid-dosage submissions as well as various matrices such as soft drinks and alcoholic beverages. This presentation will highlight the data and tests conducted to validate the hydroxamate colorimetric spot test for the detection of GHB and GBL in different matrices.

This work was conducted in order to determine how well the ferric hydroxamate test would be able to detect GHB and GBL in a variety of matrices including beverages. The procedure is a four-step procedure: (1) addition of 1 drop of concentrated H_2SO_4, (2) 3 drops of 0.5M hydroxylamine HCl in 95% ethanol/6M NaOH, (3) 1 drop of concentrated HCl, (4) 1 drop of 5% $FeCl_3$. Treatment of solutions of GHB and/or GBL with the reagents in this successive procedure produces a deep magenta color immediately. The magenta color formed with a positive reaction was distinctly different from the color of a negative reaction, which produces a brown precipitate or a ferric chloride solution, which was light yellow in color. Using commercially purchased synthetic GHB in pure deionized water, the hyrdroxamate color test gave a positive magenta color response down to 1 mg/mL. No false negatives were observed. All tests were conducted on the matrix, a water blank (deionized H_2O, concentrated H_2SO_4, and reagents), reagent blank (reagents only), and a matrix blank (matrix, reagents, and concentrated H_2SO_4). Potential interferents and water gave a similar response. Experiments with beverages containing GHB focused on allowing spiked and unspiked beverages to be compared directly.

Matrices tested included water (tap and bottled), coffee (regular and decaf), cranberry juice, orange juice, sprite, coca-cola, lime juice, lemon juice, pineapple juice, an energy drink, mouthwash, and various alcoholic beverages including several different wines, beers, and liquors. Analytes tested were GHB, GBL, 1,2-butanediol, 1,3-butanediol, 1-4-butanediol, 2,3-butanediol, beta-butyrolactone, gamma-valerolactone, caprolactone, dihydrocoumarin, dextromethorphan hydrobromide, caffeine, ephedrine, papaverine, cocaine, diazepam, and methamphetamine. It should be noted that the only other compounds tested that gave a false positive were other lactones (gamma-valerolactone caprolactone, dihydrocoumarin, and betabutyrolactone). This is to be expected since the test is specific for lactones, but is not of concern since these compounds are normally not encountered in GHB submissions. Of particular note, the test is negative for alcoholic beverages tested except for the red wines which were difficult to interpret due to the color of the solution.

The color formation with the ferric-hydroxamate reagents constitutes a highly specific screening test for GHB and/or GBL in a variety of matrices normally encountered in a forensic environment. The low cost of the reagents and their apparent reliability suggest that the test would be a useful screening tool for forensic scientists in the crime laboratory as well as officials investigating rape cases.

References:

1. K Michalik and TA Brettell, Abstract #B71, 60th Annual Meeting of the AAFS, Washington, DC, February 22, 2008.

WC Alston and K Ng Forensic Sci. Int. 2002; 126(2002):114-117.

Forensic Science, GHB, GBL

K51 Quantitative Mass Spectrometric Imaging of Drugs of Abuse in Postmortem Human Brain Tissue

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After attending this presentation, attendees will understand how to perform direct detection and quantitation of drugs of abuse in intact tissues by using deuterated internal standards and matrix-assisted laser desorption/ionization tandem mass spectrometry (MALDI-MS^n).

Direct quantitative mass spectrometric imaging of intact tissue will impact the forensic science community by providing an alternative approach to conventional drug analysis in tissue, which typically involves tissue homogenization, resulting in loss of histological information for drug distribution.

The ability to measure the regional distribution and concentration of drugs of abuse and their metabolites in postmortem brain tissue of chronic human drug users would be an invaluable tool in determining the pharmacological and toxicological actions of the drug of abuse in the human brain. Conventional drug analysis in tissue involves homogenate preparation, followed by extraction and/or derivatization. The extracts are then analyzed by gas chromatography/mass spectrometry (GC/MS) or liquid chromatography/mass spectrometry (LC/MS). Sample pretreatments are known to introduce variation in detection. The preparation of tissue homogenate precludes the opportunity to acquire detailed histological information for drug distribution. Mass spectral imaging using MALDI-MS^n provides an alternative approach for the quantitative imaging of drugs of abuse in human brain tissue, while keeping the physical features of the autopsied brain intact.

Tissue samples were excised from the nucleus accumbens (a dopamine-rich area of the striatum) and were snap frozen in liquid nitrogen and stored at -80°C. The drug of abuse and its deuterated analog (internal standard) were spiked onto 20-µm tissue slices using a micropipet. Using an artistic airbrush, MALDI matrix was applied to the tissue. The distribution of the drug of abuse in tissue was imaged using a linear ion trap with intermediate-pressure MALDI source. A MS^n isolation window was selected to include the $[M+H]^+$ ions of both the drug of abuse and the internal standard. An average of ten laser shots per scan was used to produce mass spectra.

Experiments show that ratioing the peak intensities of the analyte and a deuterated internal standard reduces shot-to-shot variability, which is due, in part, to nonhomogeneous crystallization of the matrix on tissue. The MALDI matrix for each drug analysis was chosen based on its ability to ionize the analyte efficiently and to minimize interfering ions. 2,5-Dihydroxybenzoic acid was chosen for the analysis of cocaine and its metabolites; α-cyano-4-hydroxycinnamic acid was determined to be the optimal matrix for the analysis of 6-monoacetylmorphine and morphine.

Brain tissue is a complex sample environment containing a multitude of endogenous lipids and other species that can act as interferants. MS^n methods were developed to increase the selectivity and sensitivity for the target drug analytes in brain tissue. MS^n parameters were optimized for the $[M+H]^+$ ions of cocaine, benzoylecgonine, ecgonine methyl ester, cocaethylene, 6-monoacetylmorphine, morphine, and the corresponding trideuterated analogs of these species. Instrument software allows for only one isolation window in MS^n experiments, isolating one parent mass (or range of masses) for collision-induced dissociation (CID). This means that MS^n of the target ions of the analyte and internal standard would typically be performed with two separate MS^n experiments. This would increase the response variability and counteract the signal normalizing effects of using an internal standard. Using a 6-amu-wide isolation window centered at a mass-to-charge between the $[M+H]^+$ ions of the drug analyte and its deuterated analog allows for isolation and CID of both ions during a single MS^n experiment. This single isolation method reduces the signal variability inherent with MALDI compared to isolating each ion individually with a 1-amu window (in two alternating MS^n experiments). This method was used to detect and quantitatively image drugs of abuse and their metabolites in postmortem human brain tissue.

This study demonstrated that MS^n increases selectivity, which is critical for differentiating analyte ions from matrix ions and endogenous compounds found in brain tissue. It was also shown that the use of internal standards corrects for signal variability in quantitative MALDI arising from inhomogeneous crystal formation, inconsistent sample preparation, and laser shot-to-shot variability. Using a single MS^n experiment with a wide isolation window to isolate both analyte and internal standard target ions provided improved precision (10-20 times reduction in %RSD) for quantitative imaging studies compared to using two alternating MS^n experiments that isolate the analyte and internal standard target ions separately.

Mass Spectrometric Imaging, Drug Quantitation, Brain Tissue

K52 6-Monoacetylmorphine Confirmation by Liquid Chromatographic Tandem Mass Spectrometric Determination of 6-Monoacetylmorhine and Noscapine in Vitreous Humor

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After attending this presentation, attendees will learn of the great care required in the interpretation of low level determinations by liquid chromatographic tandem mass spectrometric (LC/MS/MS) methodologies.

This presentation will impact the forensic community by demonstrating how LC/MS/MS is an extremely sensitive technique and extraordinarily low levels of drugs are routinely detected. Interpretation at these extremely low levels is not straight-forward.

The objective of this work is to present a potential pitfall in modern tandem mass spectrometric methodologies. Tandem mass spectrometry is an extremely sensitive technique and extraordinarily low levels of drugs are routinely detected. Interpretation of these extremely small amounts of drugs or metabolites is not straightforward. For example, substances with similar retention times and the same ion transitions can potentially result in false positives, as has been observed in the liquid chromatographic tandem mass spectrometric (LC-MS/MS) analysis of succinylcholine and venlafaxine. These instances illustrate a potential limitation of typical multiple reaction monitoring (MRM) techniques in qualitative confirmations of these non-routine analytes.

It has been observed that a similar deficiency may occur in the LC-MS/MS identification of 6-monoacetylmorphine (6-MAM), a commonly accepted marker of heroin abuse. On occasion, it has been observed LC-MS/MS peaks with retention times and ion transitions very similar to 6-MAM have appeared in specimens containing morphine but not anticipated to involve heroin.

The veracity of MRM identification of this "6-MAM" was examined by simultaneously determining the presence of 6-MAM and noscapine in vitreous humor. Noscapine is an alkaloidal substance, found in the opium poppy. It persists throughout the manufacture of heroin and it, and other similar alkaloids, have been proposed as urinary markers of heroin usage.

Morphine, 6-MAM, and noscapine was examined in vitreous humor from a series of twelve morphine-positive cases. Two of the cases were thought to not involve heroin, although 6-MAM had been putatively identified in stomach contents by LC-MS/MS. Morphine was confirmed in all of the vitreous specimens, 6-MAM in ten, and noscapine in eight. All of the noscapine-positive specimens also contained 6-MAM. Neither 6-MAM nor noscapine were detected in vitreous from the two cases not expected to involve heroin, although 6-MAM had previously been "detected" by LC-MS/MS in stomach contents.

Noscapine was employed as an alternate indicator of heroin use and suggest that 6-MAM might be falsely identified by typical MRM techniques. This suggests that LC-MS/MS identifications based on a small number of ion transitions are fallible, and that great care must be taken during the interpretation of detecting 6-MAM at low levels.

6-Monoacetylmorphine, Noscapine, LC/MS/MS

K53 National, Regional, and Local Patterns of Drugs Seized by Law Enforcement and Analyzed by Crime Laboratories in the United States: 2004 - 2007

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After attending this presentation, attendees will better understand the complexity and geographical variation of the U.S. drug problem and will also recognize the contributions of forensic laboratories and scientists not only to drug law enforcement issues but also to providing key scientific data for drug policy initiatives. This presentation will focus on the "supply side" of the drug problem by addressing the issue of the distinct drugs seized by law enforcement agencies and analyzed by over 275 of our nation's crime laboratories.

The presentation will impact the forensic community by acknowledging the large contribution of crime laboratory forensic scientists. The presentation will also contribute to a clearer understanding of varying dimensions and components of drug trafficking and abuse of both licit and illicit drugs.

Our nation's drug problem consists of patterns of trafficking, consumption, and diversion of both licit controlled drugs and illicit drugs that vary across time and location. Data from DEA's National Forensic Laboratory Information System (NFLIS) will be presented to depict key issues concerning national, regional and local drug problems. State and local forensic laboratories analyze substances secured in law enforcement operations across the country and offer a valuable resource for monitoring and understanding drug abuse and trafficking, including the diversion of legally manufactured drugs into illegal markets. During the period January 2004 through December 2007, an estimated 7,227,531 drug items were analyzed by state and local laboratories in the United States. The number and percentage of the top five controlled prescription drugs and the top four illegal drugs analyzed during 2004-2007 will be presented at national and regional levels (see table below). The distribution of these drugs across state and metropolitan areas will also be examined.

	National		West Region		Midwest Region		NE Region		South Region	
Illegal Drugs	*Number*	*%**	*Number*	*%**	*Number*	*%**	*Number*	*%**	*Number*	*%**
Cocaine	2,363,308	32.70	283,498	20.32	453,621	25.93	464,081	37.85	1,162,108	39.78
Cannabis/THC	2,371,585	32.81	324,365	23.25	792,232	47.03	367,070	29.93	887,915	30.39
Methamphetamine	848,495	11.74	512,157	36.71	122,488	7.27	6,127	0.50	207,723	7.11
Heroin	372,141	5.15	49,342	3.54	82,095	487	128,437	10.47	112,265	3.84
Prescription Drugs	*Number*	*%**	*Number*	*%**	*Number*	*%**	*Number*	*%**	*Number*	*%**
Hydrocodone	109,440	1.51	11,518	0.83	17,931	106	13,819	1.13	66,176	2.27
Alprazolam	108,734	1.50	**	**	21,124	125	14,746	1.20	66,791	2.29
Oxycodone	92,764	1.28	10,886	0.78	18,393	109	27,566	2.25	35,919	1.23
Methadone	33,882	0.47	4,767	0.34	5,355	032	8,564	0.70	15,197	0.52
Clonazepam	29,426	0.41	2,872	0.21	6,489	039	8,601	0.70	11,464	0.39

* Percent columns represent percent of estimated total drug items analyzed in the period January 2004 - December 2007

** Estimates for this drug do not meet standards of precision & reliability - too few laboratories reported this specific drug

Highlighted findings will include the prevalence of drugs seized and analyzed with special emphasis on controlled drugs such as opioid analgesics and benzodiazepines. Geographic Information System (GIS) generated maps will be used to display levels of seized drugs identified based on the "county of seizure" for a representative state from each of the census regions. The distribution of major drug categories across states as well as drugs identified in strategic locations will also be presented. The integration of GIS functionality for data exploration and display further enhances the importance of the NFLIS data as an informational resource for drug policy and drug control agencies by providing timely information on drug trafficking and abuse spatial patterns across the United States.

Drug Seizures, Drug Database, Geographic Information System (GIS) Display

K54 A Nuclear Magnetic Resonance (NMR) Based Study of Urine Samples Containing Drug of Abuse: Scope and Limitations of the Technique

Gloria Proni, PhD, John Jay College of Criminal Justice, 445 West 59th Street, Science Department, 10019, New York, NY ; Donna K. Wilson, MS, 570 Saint Marks Avenue, #3, Brooklyn, NY 11216; and Elise Champeil, PhD, John Jay College of Criminal Justice, 445 West 59th Street, New York, NY 10019*

After attending this presentation, attendees will learn about the use of NMR spectroscopy in drug of abuse detection.

This presentation will impact the forensic science community by exploring a new spectroscopic technique for the analysis of forensic samples.

Testing for substances of abuse in urine has great forensic relevance. The need for testing arises in many different situations: identifying drugs of abuse, supporting or denying a person's statement that they have or have not taken drugs, or determining what drug may have caused an overdose. In the following presentation, the advantages and limitations of using nuclear magnetic resonance (NMR) spectroscopy for the identification of substances of abuse in urine is

explored. Opioids were chosen for analysis as federal drug testing in urine mandates a higher cutoff level than other substances of abuse. NMR spectroscopy is the method chosen for the analysis on the basis of many advantages: it allows positive identifications of chemically different species (very similar substrates can be usually identified); very little sample preparation or operator training is needed; and, spectra could be gathered in very short times.

Codeine, morphine, and oxycodone were used in this study. Initially, these compounds were dissolved in an artificial urine solution of ten components formulated to model the NMR spectrum of real urine and NMR spectra were recorded. Later, real urine and forensic samples from deceased patients were used in the investigation.

From preliminary data, NMR spectroscopy has proven to be a novel, feasible, and useful technique for the study of opioids in urine samples. The three opioids, which present very similar structures, could be distinguished from one another in both water and artificial urine. Moreover, all three drugs could be identified at a concentration of 2000 ng/ml, equal to the federal cutoff limit given by the United States Department of Health and Human Services. This was easily done with a simple analysis of chemical shift differences. These characteristic peaks were observed at low concentrations suitable for drug testing. These peaks, arising from two protons on the phenyl group of phenanthrene opioids, were found between 6 and 7 ppm. For morphine, the difference in frequency was near 41 Hz while for codeine is near 71 Hz. For oxycodone, the difference in frequency is near 43 Hz. The ease of use NMR instrumentation, speed of analysis, as well as the small sample amount needed and the fact that is a non-destructive technique render NMR spectroscopy an advantage over current forensic methods used to analyze substance of abuse in urine.

This presentation was supported by John Jay College of Criminal Justice 2007 John Jay Research Assistance Fellowship and the PSC-CUNY award 60035-37 38.

NMR, Opioids, Urine

K55 Sample Preparation of Cannabiniods in Urine Using Dispersive Solid Phase Extraction and Clean Up

Jack Cochran, BS, and Kristi Sellers, BS, Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823*

After attending this presentation, attendees will understand how to increase sample throughput using a simplified sample clean up method for analyzing cannabinoids in urine as well as understand how to derivatize and analyze cannabinoids by GC-MS.

The sample preparation and analysis methodology discussed will impact the forensic community by providing an alternate means of processing and analyzing cannabinoids compared to current sample preparation and analysis methodologies.

The main psychoactive component in marijuana, Δ^9–tetrahydrocannabinol (Δ^9–THC), is quickly absorbed and metabolized to 11-hydroxy-Δ^9-tetrahydrocannabinol (hydroxy-THC), an active metabolite. The hydroxy-THC is further metabolized (rapidly) to 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (carboxy-THC), an inactive metabolite commonly found in urine, blood, hair, and other tissues. GC-MS (Gas Chromatography-Mass Spectrometry) often is used for confirming and quantifying Δ^9–THC and carboxy-THC. However, GC-MS methods require time-consuming steps like sample clean up to obtain acceptable chromatography. Using a dispersive solid phase extraction and clean up technique saves time without sacrificing reproducibility and sensitivity.

This study included developing a sample clean up method for analyzing cannabinoids in urine using a dispersive solid phase extraction and clean up method (dSPE). The dSPE method employs a quick extraction followed by a cleanup of the sample. Small polypropylene centrifuge tubes are prefilled with precise weights of M_gSO_4 and SPE (solid phase extraction) adsorbents to remove excess water and unwanted contaminants from the samples. After agitation and centrifugation, the cleaned extracts are ready for further processing. Samples may be derivatized, pH adjusted to protect sensitive compounds and/or solvent-exchanged to improve analysis by GC-MS. Internal standards can also be added. The samples are then ready for analysis by GC-MS. Also, dSPE and sample clean up process can be used for HPLC-MS (high performance liquid chromatography-mass spectrometry) applications.

A reproducible, quantitative GC-MS method for analyzing cleaned-up, derivatized cannabinoids in urine was developed. Two goals were the focus in this study: (1) to reduce sample clean up time for cannabinoids in urine, and (2) to provide a reliable and reproducible sample preparation method for quantification data in the low ng range (< 10ng). Compounds analyzed for were Δ^9–THC and carboxy-THC. The internal standard used was deuterated THC. Derivatizing reagents experimented with included a silylation reagent and an acylation reagent. The instrument used was a Shimadzu GC-MS.

Results showed that the proper dispersive solid phase extraction and sample clean up method coupled with the proper derivatization reagent produced reproducible data with linearity across a broad range of concentrations. The limit of detection (LOD) reached was as low as 5ng on-column, and sample preparation time was reduced. The use of GC-MS allowed for identification of derivatized cannabinoids, particularly Δ^9–THC and carboxy-THC, relative to their unique mass spectra. Analysis time was kept under 10 minutes since run conditions were optimized.

In conclusion, the methods developed in this study can benefit analysts by providing a simple and short extraction and clean-up procedure, by providing a reproducible derivatization procedure and by providing reduced analysis times using GC-MS for cannabinoids in urine.

Sample Preparation, GC-MS, THC

K56 A Fast GC/MS Method for the Analysis of Common Selective Serotonin Reuptake Inhibitors

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After attending this presentation, attendees will learn about a validated, fast method for routine analysis of common selective serotonin reuptake inhibitors (SSRI's) in human urine.

This presentation will impact the forensic community by demonstrating how choosing the right combination of column and carrier gas results in significant improvement in analytical procedures and improves throughput in a routine testing laboratory.

SSRI's included in this method are: fluoxetine, norfluoxetine (fluoxetine metabolite), sertraline, norsertraline (sertraline metabolite), citalopram, and paroxetine. Deuterated paroxetine (paroxetine-D6) was used as the internal standard.

Urine samples were hydrolyzed using β-glucuronidase from *Escherichia coli,* centrifuged for 5 minutes and then approximately 1 gram of a salt mixture (sodium chloride, sodium carbonate and sodium bicarbonate, 6:1:1 by weight) was added. The alkalinized urine specimens were extracted using liquid-liquid extraction with heptane/ dichloromethane/ dichloroethane/ isopropyl alcohol (10:5:5:1). The organic upper organic layer was separated and dried under air at 40°C. The dried sample extracts were derivatized for 10 min at 65°C with MSTFA/ammonium iodide/ ethanethiol reagent (50 mg/25 mL/75 µL).

GC/MS analysis was performed in electron ionization mode by selective ion monitoring (EI - SIM) using a single quadrupole mass spectrometer with inert ion source. A 230 volt GC oven was used to enable fast temperature programming and hydrogen was used a carrier gas. Separation was performed on a narrow bore column (10 m X 0.15 mm i.d.). All analytes were eluted within 4.5 minutes with Injection-to-injection analytical run time of 7.5 minutes. Three ions for each analyte; paroxetine (249.1, 264.1, 401.2), Fluoxetine (219.0, 262.2, 381.2), norfluoxetine (174.1, 320.1, 439.2), sertraline (274.0, 276.0, 377.1), norsertraline (274.0, 276.0, 320.0), citalopram (238.1, 324.1, 208.1), and two ions for the internal standard; paroxetine- D6 (252.1, 270.2) were monitored.

The procedure was applied to authentic urine specimens and the results showed that hydrolysis is essential to the optimum recovery of most analytes. All analytes were successfully detected in the 4.5 minute run time utilized. The limit of detection for all analytes was 100 ng/mL except citalopram, for which it was 50 ng/mL. The limit of quantitation for citalopram and paroxetine was 100 ng/mL and for all other analytes it was 150 ng/mL. Precision was within 6% and quantitative accuracy was over 94% for all analytes. The method was linear up to 20,000 ng/mL for paroxetine and upto 2000 ng/mL for all other analytes.

A fast and simple GC/MS method was developed for the routine analysis of common SSRI's in urine. This method can easily be used for other body fluids such as blood. The simple sample preparation, combined with short, narrow bore GC column and hydrogen as a carrier gas, drastically decreased sample turnaround time and increased throughput without compromising sensitivity or selectivity.

SSRI's, GC/MS, Hydrolysis

K57 Evaluating the Presence and Dangers Associated With Heavy Metals in Commonly Encountered Consumer Products

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After attending this presentation, attendees will learn how to develop a new method for evaluating the presence of heavy metals and their transfer into human saliva.

Heavy metal contamination is a significant problem for consumer products imported from outside of the United States. This presentation will impact the forensic community by evaluating its regional prevalence and shedding light on a new method to evaluate the associated hazard.

Since the late 1970's lead levels in commercially available paints have been regulated in the United States by the Consumer Product Safety Commission (CPSC). Levels of lead above 600 ppm were banned on surface coatings, toys, and other items intended for children, and furniture. While this has been successful in regulating products made in this county, an unintended consequence of the global economy is that more of these regulated items are being produced outside of the U.S. For the most part, the CPSC relies on the foreign manufacturer to comply with these U.S. guidelines. Recently this self-regulation has resulted in the CPSC issuing numerous product recalls. Over 42 million toys have had to be removed from the market due to excessive lead paint contamination of the toy's surface. With clear published standards on lead levels, investigations into how these items were allowed on the market might well result in criminal charges.

While lead's toxicity is well established and its presence well regulated, other unregulated heavy metals found in paints as pigments or driers might also pose health risks. This preliminary study evaluated the presence of heavy metals in commonly encountered consumer products that either by design or by chance; ends up in an individual's mouth. The most obvious candidate for this category is children's toys. While it is usually not safe, it is common for young children to put non-food items in their mouths. A number of children's toys including some that had been subjected to a CPSC lead paint recall were evaluated as part of this study. Samples were not strictly limited to children's toys but also included some items that adults might inadvertently put in their mouths. To this end, pencils, pens, and certain cosmetic items were also evaluated.

Samples evaluated in this study were first screened for the presence of heavy metals. Initially this was accomplished through the use of Scanning Electron Microscopy with Energy Dispersive Analysis (SEM EDS). This approach proved problematic so screening was then changed to a portable x-ray fluorescence (XRF) unit which allowed for rapid non-destructive real-time elemental analysis.

Currently there is an abundance of literature regarding lethal doses of heavy metals and their associated toxicities. What's lacking are any studies that demonstrate how much of these metals are transferred into human saliva, the matrix that these samples would be exposed to. To address this issue an experiment was designed where a fixed amount of solid lead was placed into a set volume of human saliva that was maintained in a conical tube at body temperature. The saliva was then sampled at intervals from 30 seconds up to 240 minutes and compared against a saliva blank maintained under the same conditions via atomic absorption spectroscopy (AAS). Statistically significant concentrations of lead were found after x minutes that remained reasonably consistent for the duration of the exposure. This was performed for each heavy metal that was being evaluated by AAS.

Samples that had positive screening results with the XRF were then evaluated through the saliva transfer test. A 1 cm square was excised from the sample and allowed to sit in human saliva maintained at body temperature for XX. Results were then compared with saliva blanks also maintained at temperature. The Excised samples were then removed from the saliva, mechanically broken down, and then replaced into the saliva again. After being allowed to sit for X minutes again, the saliva was revaluated for the presence of the heavy metal.

Metals, Transfer, Saliva

K58 Postmortem Pediatric Toxicology

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After attending this presentation, attendees will be better prepared to interpret postmortem pediatric cases when there are toxicological findings. Attendees will understand the fundamental differences between adults and children in respect to toxicological findings.

This presentation will impact the forensic science community by broadening and deepening the knowledge base around the role of toxicants in postmortem pediatric cases.

In this 10th Annual Special Session within the Toxicology section, pediatric cases involving toxicological findings are discussed. As a relative dearth exists of interpretive information involving toxicological findings in the pediatric population, this session is a forum to help elucidate and clarify such issues. The format is a short case presentation

including pharmaco-toxicokinetic data and other relevant ancillary information followed by audience participation to provide interpretive clarity around the case-specific impact of the toxicological findings.

This year's presentations will be by:

Dr. Andrew Baker, Chief Medical Examiner, Hennepin County Medical Examiner, Minneapolis, MN will present a case-specific issue involving the role of a toxicant in a pediatric death.

Dr. Dan Isenschmid, Chief Toxicologist, Wayne County Medical Examiner's Office, Detroit, MI will discuss a case highlighting the concept that "Children Are Not Small Adults" through discussion of single dose administration of oxycodone.

Dr. Karen Ross, Assistant Coroner and Pathologist, Jefferson Parish Forensic Center, Harvey, LA will discuss a case highlighting the continued concerns of over-the-counter (OTC) preparations and exposures in children. Despite the FDA's mandate to remove OTC infant preparations, it is doubtful that the use of such preparations in this population will stop. Recognition of safety concerns with these products in children far exceeds the relatively recent media attention given the subject.

Dr. Marina Stajic, Chief Toxicologist, Office of Medical Examiner, New York, NY, will bring forward an unusual case involving fentanyl in a child. As a potent opioid with a narrow therapeutic index, fentanyl represents both a highly effective pain medication and a potential significant contributor to toxic sequelae. The illicit exposure of children to this compound is of significant concern due to its toxicological properties.

Pediatric, Toxicology, Postmortem

TOXICOLOGY

K1 Gas Chromatography of Postmortem Blood Revealing Sevoflurane in a Patient Six Hours Post-Op

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Attendees will understand basic physiology and properties of sevoflurane, a general anesthetic used in same-day surgeries. Attendees will also learn that sevoflurane may interfere with an ethanol peak with a certain method of gas chromatography.

This poster will impact the forensic science community by alerting the community to the possibility of the presence of "ethanol" peaks on gas chromatography due to sevoflurane in post-operative patients.

A 54-year-old female patient underwent facelift surgery which lasted approximately six hours. During surgery, sevoflurane was used for induction and maintenance of anesthesia. There were no intraoperative complications. The patient was discharged home about one hour and forty-five minutes post-op at 1645 hours. Her only post-op complaint was of a migraine headache, which was treated with topiramate (Topamax®). Patient history as detailed by her family, stated that the patient consumed only ginger ale and yogurt prior to going to sleep after surgery. At 2100 hours, she was sleeping soundly. At 2115 hours, she was not breathing and unresponsive. Postmortem examination revealed focal moderate calcific atherosclerotic narrowing of the proximal left anterior descending coronary artery, microscopic fibrosis of the superior interventricular septum of the heart near the atrioventricular node, and mild to moderate microvesicular steatosis of the liver. Postmortem toxicology revealed the presence of citalopram (Celexa®), lidocaine, morphine, and fentanyl. Gas chromatography (GC) of postmortem blood revealed a peak at 2.496 seconds retention time, consistent with ethanol (retention time 2.3 ± 0.1 sec). The concentration of ethanol was calculated to be 0.05 g/dL. Antemortem blood also revealed ethanol by GC at a concentration of 0.04 g/dL. However, the vitreous fluid was negative for ethanol.

Due to the family's insistence that the patient had not consumed ethanol, possible interferences were sought. Of the medications the patient received, sevoflurane was the best possible medication to cause interference. Sevoflurane [fluoromethyl 2,2,2,-trifluoro-1-(trifluoromethyl) ethyl ether] is a four carbon molecule that exists as a liquid and is used for induction and maintenance of anesthesia. A review of the literature revealed two manuscripts which reported ethanol and sevoflurane peaks to be within 0.1 and 0.15 seconds of each other (Biomed Chromatogr 2004; 18: 714-18 and Clin Chem 2001; 47: 281-91, respectively). Ethanol-negative blood was spiked with varying dilutions of sevoflurane, which co-eluted with ethanol. For example, the retention time of the 1:20,000 dilution was 2.483 seconds. Volatile analysis was performed with a head space procedure, using *n*-propanol as the internal standard. The column was a 6 foot Porapak-S at a temperature of 180°C. Instrumentation was Shimadzu GC-I 4A, Kyoto, Japan. Due to the high volatility of sevoflurane, a linear concentration curve could not be produced.

A study by Kharasch et al. of sevoflurane's metabolism and pharmacokinetics shows that it can still be detected in a patient's blood several hours after an administration of three to five hours (Anesthesiology 1995; 82: 1369-78). The average half-life of sevoflurane in that study was 2.8 ± 1.0 hours. The above patient died approximately six hours after the end of anesthetic administration. The long detection time may be partially due to the high partition coefficient for adipose tissue. Adipose tissue dominates the pharmacokinetics past three hours post-administration (BMC Clin Pharmacol 2007; 7:1-21). The above patient had a body mass index of 28.6 kg/m^2, indicating a possible increase in body fat percentage over normal.

Therefore, although ethanol cannot be completely excluded, it is likely that the above patient did have sevoflurane in her blood, causing an interfering peak on gas chromatography. An interfering peak was obtained when ethanol-negative blood was spiked with sevoflurane. However, due to the high volatility of sevoflurane, consistent data points could not be obtained to determine the concentration of sevoflurane in the patient's blood.

Gas Chromatography, Sevoflurane, Ethanol

K2 Signature Analysis of 25 Illicit Cocaine Samples and a Comparison to Analysis by AccuTOF™ DART™

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After attending this presentation, attendees will gain an enhanced understanding of multiple techniques for characterization of illicit cocaine and their ability to assess purity, level of performance, and their ability to determine specific compounds of interest.

This presentation will impact the forensic science community by providing a comparison of established quantitative, chromatographic method to a novel qualitative time-of flight mass spectrometric method to determine the purity of illicit cocaine and its relative abundance of signature compounds.

Introduction: Characterization of 25 illicit cocaine samples was undertaken in support of a research project funded in part by the National Institute of Justice (NIJ Award # 2006-DN-BX-K019) examining the ratios of cocaine-related compounds in hair samples contaminated with cocaine. Various coca-related compounds, isotope ratios and solvent determinations among other parameters are used to determine the manufacturing process and geographical origin of cocaine exhibits. Furthermore, this information is a useful tool to answer questions related to cocaine distribution and trafficking.

A novel application of direct analysis in real time (DART) sample introduction coupled with time-of-flight (TOF) mass spectrometry was evaluated for analyzing 25 samples of bulk powdered illicit cocaine hydrochloride salt seized by the Drug Enforcement Administration (DEA). The cocaine samples were analyzed by the DEA to determine their "signature" including their purity and the presence of specific compounds including products of manufacture, adulterants, and other cocaine analytes including oxidation products. The results of the analysis were then compared to data obtained by CFS to assess the AccuTOF-DART's level of performance.

Methods: Analysis was conducted in positive mode using AccuTOF-DART mass spectrometry. After analysis of the cocaine samples, each data set was examined for the presence of cocaine analytes (e.g., cocaine, benzoylecgonine, ecgonine ethyl ester, cocaethylene, norcocaine, anhydroecgonine methyl ester, truxillines, other ethyl esters) and a number

of compounds typically found in illicit cocaine. These compounds include methyl isobutyl ketone (MIBK), methyl ethyl ketone (MEK), ethyl acetate, n-propyl acetate, isopropyl acetate, mannitol, and petroleum ethers. Data obtained from both DEA signature analysis and AccuTOF-DART were compared to evaluate the level of performance of the AccuTOF-DART. Samples were also submitted to the Armed Forces Institute of Pathology (AFIP) for a limited GC-EI-MSD method as an additional confirmation of the norcocaine content.

Results: The AccuTOF-DART analysis of the cocaine samples resulted in the detection of the analytes anhydroecgonine methyl ester (AEME), tropacocaine, and trimethoxycocaine. Although AEME was easily detected, tropacocaine and trimethoxycocaine were detected intermittently, as were truxillines and MEK. In most samples, there was an ion present at 290.151 m/z, which is the M +H value of $C_{16}H_{19}NO_4$. This ion is consistent with both benzoylecgonine and its isomer norcocaine which have indistinguishable accurate masses. The table below shows the number of illicit cocaine samples in which the various components were detected by the three analytical processes (TOF-DART, DEA signature analysis, and AFIP GC-EI-MSD).

Using TOF-DART, further testing would be required under a different set of instrument parameters in order to distinguish the presence of norcocaine and/or benzoylecgonine. In these 25 cocaine samples, only trace quantities of cocaethylene were detected by DEA signature analysis.

Conclusions: This study has demonstrated the AccuTOF-DART's ability to analyze cocaine quickly and effectively. The TOF-DART is an adequate screening tool, but it does not currently have the level of performance required for purity calculations. The AccuTOF-DART provided some, but not all of the signature compounds determined to be present in the samples analyzed by other established methods. Within these 25 cocaine samples, one cocaine sample had norcocaine present at approximately 8% when compared to the cocaine. However, because of the intermittent detection of some of the analytes, variables such as sampling and instrument parameters need to be further investigated. Based on these samples, the DART-TOF would be a useful tool for the screening of samples and in some but not all circumstances may provide conclusive determination of chemical identity. These data will be used for future contamination studies in hair performed at RTI.

Technique	AEME	BE	CE	NCOC	Trimethoxy-cocaine	Tropacocaine	Truxillines	Total Cinnamoyls
TOF-DART	22	25*	ND	25*	5	7	25	ND
DEA	ND	21	7	21	25	25	25	25

* NCOC and BE indistinguishable by TOF-DART.

Cocaine, TOF-DART, Analysis

K3 Macronutritional Composition Induced Differential Gastrointestinal Absorption Kinetics of Alcohol: A Pharmacokinetic Analysis of Alcohol Absorption in the Postprandial State

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After attending this presentation, attendees will gain an understanding of the variable impact that a meal induces on the absorption rate of alcohol based on the macronutritional composition of the food—the nutritional components of food that provide calories or energy (proteins, fats, carbohydrates).

This presentation will impact the forensic community by providing additional knowledge to be used during interpretation on the rate that alcohol is absorbed in the postprandial state.

The current method of assessing gastrointestinal absorption of alcohol in the postprandial state include both the size of the meal as well as the time lapse since the meal was consumed relative to the ingestion of alcohol. It is hypothesized that an ingestion of alcohol following a meal will display differential gastrointestinal absorption rates dependent upon the macronutritional composition of the meal. It is further hypothesized that the glycemic index (GI)—a measurement of the magnitude and rate at which ingested food causes the level of glucose in the blood to rise—can be utilized as a third parameter in the absorption rate constant when predicting gastrointestinal absorption kinetics.

These hypotheses were investigated in a two-day study distinguished by the prandial state of five healthy, non-alcoholic male volunteers (four Caucasian and one African American) with mean age 24y (range 21y to 27y), mean height 186cm (range 183cm to 190cm), and mean weight 90kg (range 63kg to 114kg) who were on no medication and had no evidence of gastrointestinal disease. Informed consent was obtained through Georgia Public Safety Training Center study protocols for conducting controlled alcohol drinking studies. Volunteers were instructed to restrict any food consumption for at least three hours prior to the study on each day. Volunteers were administered five separate diluted (diet soda, caffeinated) ingestions of alcohol (40% alc/vol) for a total mean consumption of 0.80g/kg (range 0.68g/kg to 1.00g/kg) ethanol over a course of two hours. Alcohol administration schedule and net weight remained constant for the respective volunteer on each study day. Day one assessed the absorption kinetics in the fasting state for all volunteers. On day two, the volunteers were separated into three groups dependent upon the type of meal administered: Group A) High GI (two volunteers) – 350kcal total [72g carbohydrates (0g fiber), 10g protein, and 2.5g fat]; Group B) Medium GI (one volunteer) – 352kcal total [36g carbohydrates (0g fiber), 43g protein, and 4g fat]; and Low GI (two volunteers) – 349kcal total [0g carbohydrates (0g fiber), 76g protein, and 5g fat]. For each study day, the breath alcohol concentration (BrAC) was recorded on a set schedule (90min, 150min, and 210 min elapsed time from scenario beginning) using the Intoxilizer 5000.

Results of the study revealed that all volunteers displayed similar absorptive kinetics and peak BrAC on study day one in the fasting state (volunteer # [1] 0.099 g/dl, [2] 0.106 g/dl, [3] 0.106 g/dl, [4] 0.124 g/dl, [5] 0.111 g/dl). On study day two, in the postprandial state, all volunteers displayed attenuated, but differential peak BrAC, dependent upon the assigned group. Group A) High GI – volunteers (#[1] and [2]) exhibited a mean decreased peak BrAC of 13% (range 8% to 17%). Group B) Medium GI – the volunteer (#[3]) exhibited a decreased peak BrAC of 32%. Group C) Low GI – volunteers (#[4] and [5]) exhibited a mean decreased peak BrAC of 42% (range 37% to 46%).

The results of this study show that peak BrAC following a meal with low GI will be lower than following a comparable meal with similar calories but higher GI. This finding provides significant insight as to the effect the macronutritional composition of a meal as measured by postprandial glycemia (GI) has on the absorption kinetics of alcohol in the postprandial state. Whereas high GI foods result in faster rates of gastric emptying signified by elevated postprandial glycemia and therefore increased absorption kinetics of alcohol. On the other hand, low GI foods result in slower rates of gastric emptying signified by little or no postprandial glycemic response and therefore slower absorption kinetics of alcohol in the gastrointestinal tract. Current methods of predicting postprandial absorption kinetics would suggest no intergroup variation in day two of the study where obvious variation existed. These results warrant further studies with a larger sample size including gender and ethnic variation to verify these preliminary findings.

Alcohol, Absorption, Glycemic Index

K4 Prevalence of Desmethylsertraline in Postmortem Tissue Samples

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After attending this presentation, attendees will better understand the distribution of desmethylsertraline in postmortem tissue samples and its relationship to the study of postmortem toxicology.

This presentation will impact the forensic science community and analysts/pathologists involved in postmortem undertaking toxicology investigations by making them more aware of the levels of this drug that may be found in tissue samples.

Aims: The aim of this poster presentation is to inform the forensic toxicology community about levels of the primary metabolite, desmethylsertraline, of the selective serotonin re-uptake inhibitor (SSRI) sertraline found in postmortem tissue samples. Levels of this particular metabolite are referenced to parent drug/ metabolite in blood and tissue postmortem samples.

Methods: In 2006, over 890 cases were submitted to the Forensic Toxicology Laboratory, Center for Forensic Sciences in Syracuse, NY (CFS) by the Medical Examiner's Office. In each of the cases, analyses of blood/vitreous humor, tissue (liver), and urine were performed for volatiles (GC-FID), drugs of abuse (ELISA), and weak acid neutral/ bases (GC-MS). Tissue samples were analyzed as 1: 4 homogenates of the original sample. Where sertraline was confirmed by GC-MS amongst other confirmed drugs (some 23 cases) in blood and tissue samples, quantitative analysis of setraline/ desmethylsetraline was performed employing a liquid-liquid sample extraction (n-butyl chloride/ ammonium hydroxide/ 0.1 M sulfuric acid) using certified reference standards for calibrators and controls. Chromatographic analysis was performed by GC-MS/ GC-NPD (internal standard: Mepivicaine). This was carried out according to the standard operating procedure currently in use at CFS.[1] Data from the analysis of setraline/desmethylsetraline in blood/tissue samples were collected and assessed. This information (along with other quantified drugs) was used to offer an interpretation to the office of the Chief Medical Examiner for Onondaga County to assist with the determination of cause and manner of death in forensic investigations.

Results: In this presentation, data from the sertraline/ desmethylsetraline analysis of the postmortem samples are presented. The range of desmethylsertraline in postmortem tissue samples was 0.78 mg/kg to 402 mg/kg. The corresponding range of the parent was 0.04 mg/kg to 188mg/kg. Blood levels were reported as 0.05 mg/ L to 1.51 mg/ L desmethylsertraline and 0.03 mg/L to 0.45 mg/L sertraline, respectively. In several cases i.e. # 5, 10, 14, 16, 19, and 21, respectively the levels of the primary metabolite reported in tissue samples reached 402, 51, 50, 70, 34, and 242 mg/kg, respectively. In cases like these drugs such as buproprion/ metabolite, fluoxetine/ metabolite, and tricyclic antidepressants were also present with and without the presence of ethyl alcohol.

Conclusions: Sertraline is metabolized in the liver by CYP2D6 to the primary metabolite desmethysetraline. This hepatic isoenzyme (2D6) of the cytochrome P450 group is also involved in the oxidative metabolism of other drugs. It has been reported that desmethylsetraline may accumulate in plasma due to it's slow elimation ($t_{1/2}$ 62-104 hrs).[2] In the cases presented this may be a similar effect occurring (i.e., accumulation) in the liver tissue giving rise to excessive figures. It has been reported that desmethylsetraline has only 10-20% of the pharmacological activity of the parent drug,[2] at these levels it is pertinent to ask what effect this compound on the toxicity and cause of death and its impact on postmortem drug re-distribution.

Based on data presented, toxicologists involved in the analysis of sertraline/desmethylsertraline in postmortem cases (especially tissue samples) should review all the relevant information pertaining to these cases before offering advice/ interpretation. High levels as seen in the presented cases may skew the interpretation as to a possible overdose in the assessment of cause and manner of death.

References:

1 Forensic Toxicology Laboratory Standard Operating Procedures Manual, Center For Forensic Sciences, Syrcuse NY (2006).
2 R.C. Baselt, Disposition of Toxic Drugs and Chemicals in Man 5th Ed (2000).

Desmethylsertraline, Chromatography, Toxicology

K5 Strychnine Poisoning

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The goal of this presentation is to present a case of strychnine poisoning as it is not a common or easily diagnosed.

This presentation will impact the forensic science community by demonstrating the difficulties encountered by the forensic medical doctor during investigations of homicide or suicide by strychnine poisoning.

Introduction : In Europe and North America, Strychnine is commonly known as a restricted use pesticide. In France, its use as a pesticide is forbidden. Historically, intoxications were rare and causes were generally accidental, sometimes suicidal and in limited cases homicide-related. The mechanism of neurotoxicity of strychnine is well understood and the management of strychnine poisoning is well documented. There are data about survival after strychnine poisoning and the kinetics of elimination of strychnine. Strychnine concentrations in fluid samples of fatalities have been reported. Strychnine poisoning is uncommon and often difficult to diagnose; many times toxicological analyses is performed on a few fluid samples.

Materials and Methods: This presentation reports a case of a 58 year-old man who ingested a potential poison or medication in order to commit suicide. The autopsy did not reveal a traumatic cause of the death. An autopsy was performed and samples of cardiac blood, femoral blood, gastric content, bile urine and vitreous humour were analyzed. Moreover some kind of "balls" were found in the gastric contents. From these samples, ethyl alcohol (GC) and other toxins (EIA, LC/DAD, GC/MS) were investigated in order to determine the origin of the death.

Results: Ethyl alcohol was found in urine (0.14 g/L), in gastric content (1.14 g/L) and bile (0.31 g/L). Regarding other toxins, none were found in the samples. A high concentration of strychnine in both cardiac (7 µg/mL) and femoral blood (0.64 µg/mL), in gastric content (130.5 µg/L) and bile (21.9 µg/L) was detected. A bottle containing 10 g of white powder was also received. The bottle was found on the victim's bed table. The content of this bottle was also analyzed by chromatography which confirmed the bottle contained strychnine. The death was determined to be a fatal intoxication by strychnine.

Discussion: When investigating a toxic death, strychnine intoxication is not the first hypothesis of most death investigators. A background check of the decedent is sometimes helpful in the final death determinations. In this case the man was a gamekeeper and could readily obtain strychnine. The bottle on his table confirmed the suspicion. Fluids taken during autopsy allowed for strychnine determination of several fluids that are not typically reported in the literature. Toxicology confirmed the strychnine poisoning which was recent because of the high concentration in gastric content and the low urine concentration. In the blood, the concentration was lethal (more than 10x the commonly lethal dose). In the literature, the blood is always analyzed, sometimes the gastric content and in very few occasions the urine is also analyzed.

This case demonstrates a fatal and acute strychnine poisoning with the presence of high strychnine concentrations in different fluids.

Strychnine, Poisoning, Toxicology

K6 Development of a Comprehensive Forensic Drug Information Web Site and Concentrations Database

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Upon completion of this presentation, participants will learn about: (1) a Forensic Drug Information (FDI) web site that provides access to a drug induced/drug related deaths database that allows for direct online data entry by medical examiners, (2) the use of the database to analyze drug toxicity characteristics and patterns including the influence of factors such as other drugs, patient characteristics, sample site, and time since death and other selected death investigation findings on concentrations or toxicity potential, and (3) the use of the web site to find summaries of key drug characteristics and links to Medline/Toxline abstracts involving specific drug overdose and toxicity reports.

Deaths associated with drug ingestions can be difficult to interpret for several reasons, including possible interactions or varying patient characteristics, unclear relationships of drug/metabolite concentrations to toxicity, or the influence of sample site and postmortem interval on concentrations. Although some references provide toxic and lethal drug concentration ranges, they usually involve blood or plasma and have not been established for many drugs. The Drug Abuse Warning Network (DAWN) provides valuable information about drug ingestions that result in deaths or emergency department visits; however, actual concentrations are not recorded, in addition to other limitations. This presentation will impact the forensic science community by demonstrating how the Forensic Drug Database (FDD) is designed to collect a broad range of drug and metabolite data and characterize the interrelationships among possible factors influencing toxicity. If certain patient attributes or drug combinations are found to be associated with death, educational efforts can be targeted to help prevent these types of deaths. Medical examiners can enter data from drug-induced or drug-related death cases directly into the FDD from remote locations. At present, data from over 600 cases have been entered. Simple database reports can be run from the Forensic Drug Information (FDI) web site, with participating medical examiners and coroners having complete access to the data reports.

The objectives of this project were to: (1) develop an online drug induced/related deaths database that allows for direct data entry by medical examiners, (2) classify the data using standardized DAWN terminology, (3) describe drug toxicity characteristics and patterns including the influence of factors such as other drugs, patient characteristics, sample site, and time since death and other selected death investigation findings on concentrations or toxicity potential, and (4) develop a forensic drug information web site providing database access and other features.

A Forensic Drug Information (FDI) web site (http://www.forensicdi.org) was developed that includes three main parts: (1) Forensic Drug Database (FDD) – compiles data about the drug(s), concentrations, and other relevant information found in drug-induced or drug-related death cases, (2) Database Reports – allows for online user-customized and administrator generated reports from data stored in the FDD, and (3) Literature Abstracts - contains regularly updated links directly to the Medline/Toxline abstracts of reports of specific deaths involving drugs (legal, illicit), listed alphabetically by drug name.

As of July 2007, the FDD contains over 640 cases of drug-induced or drug-related deaths compiled from the files of the West Virginia Office of the Chief Medical Examiner. Of these decedent cases, approximately 34% were female and 66% were male. The most commonly detected drugs were methadone (32% of cases), cocaine (23% of cases), diazepam (21% of cases), ethanol (20% of cases), and hydrocodone (19% of cases). Cases are continually being added to the FDD, and new medical examiners interested in contributing case data to the database are welcome (visit FDI web site to register). A variety of types of reports and statistical analyses of the drugs, decedent characteristics, and concentrations are currently in preparation. Participating medical examiners will have access to these database reports in addition to the other web site features.

*The authors would like to acknowledge and thank Lixin Wu and Nan Wu for all their work in development of the FDI web site and FDD.

Drugs, Concentrations, Toxicity

K7 A Method for the Determination of Amphetamines and Methylenedioxyamphetamines in Oral Fluid by Gas Chromatography/ Mass Spectrometry

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Upon reviewing this poster presentation, observers will become familiar with a validated GC/MS method for detecting and quantifying amphetamines and methylenedioxyamphetamines in oral fluid, which may be easily applied in a forensic drug testing laboratory. This presentation will impact the forensic science community by demonstrating how the increase in popularity of using oral fluid in forensic drug testing has provoked a need for improved and reliable methods for drug extraction, detection, and quantitation. SAMHSA is currently evaluating oral fluid (OF) in an attempt to provide a set of universal standards for laboratories. This study yields data which may be applicable to developing future SAMHSA guidelines.

The authors present the validation of a gas chromatography/mass spectrometry (GC/MS) method for the detection and quantification of amphetamine (AMP), methamphetamine (MAMP), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyehtylamphetamine (MDEA) in oral fluid. Prior to extraction, 500 uL of oral fluid was pretreated with 250 uL of 0.7M sodium periodate for 15 min. The sample was then made basic with 200 uL 1N potassium hydroxide and extracted with 1.0 mL of n-butyl chloride. After separation, the extracted amphetamines were derivatized with heptaflurobutyic anhydride (HFBA) including removal of excess HFBA by washing with 1N potassium hydroxide and water. The amphetamines were separated and quantified in an Agilent 5973 GC/MS equipped with a HP-Ultra 1, 12m X 0.2mm X 0.33um capillary column with a 4mm splitless liner. The oven temperature program was: initial 60°C for 0.2 min., then ramped at

20°C/min. to 180°C, held for 0 min., then ramped at 2°C to 185°, held for 0 min. Under these conditions the retention times in minutes of amphetamine HFBA derivatives were: AMP, 4.75; MAMP, 5.40; MDA, 6.68; MDMA, 7.54; MDEA, 7.95. The drugs were quantified with their respective deuterated species as internal standards. The MSD was operated in the SIM mode monitoring the following m/z ions: AMP-HFB, 240, 91 and 118; MAMP-HFB, 254, 210 and 118; MDA-HFB, 162, 240 and 375; MDMA-HFB, 254, 210 and 389; MDEA-HFB, 268, 240 and 403; $^{2}H_{10}$-AMP-HFB, 244 and 97; $^{2}H_{11}$-MAMP-HFB, 260 and 213; $^{2}H_{5}$-MDA-HFB, 167 and 380; and $^{2}H_{5}$-MDMA-HFB, 258 and 213; and $^{2}H_{16}$-MDEA-HFB, 274 and 244.

Amphetamine and MDA displayed a linear range 10-2000 ng/mL with a 10 ng/mL LOQ and LOD. Methamphetamine was found linear from 5-2000 ng/mL with a 5 ng/mL LOQ and LOD. The assay was less sensitive for MDMA and MDEA with a LOQ and LOD of 20 ng/mL; however, the assay was linear up to 3750 ng/mL for these analytes. The method yielded excellent precision. At a proposed cut value of 50 ng/mL and ± 25% of this cut-off (target values 37.5 ng/mL and 62.5 ng/mL), the %CV values were <5% for each amphetamine at the three target concentrations. The method was applied to specimens obtained with two different oral fluid collection devices; the Intercept (Orasure Technologies) and the Salivette (Sarstedt). A notable interference with the AMP-HFB 91 m/z ion and the MDEA-HFB 240 m/z ion was observed in oral fluid collected with the Intercept device. No interferences were observed with specimens collected by the Salivette device. The present method was found to be reliable for the determination of amphetamine, methamphetamine and their commonly abused methylenedioxy-derivatives in oral fluid.

Oral Fluids, Amphetamines, MDMA

K8 Pattern of Drug Abuse Fatalities in Teenagers

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After attending this presentation, the participants will learn the trend and pattern of drug abuse deaths among teens and will understand the urgent need to develop an effective prevention and treatment programs for teenager addicts.

Drug abuse deaths represent one of the most serious public health problems in children. These study findings show a significant increase of drug abuse deaths among teens in the State of Maryland over the past 16 years. This presentation will impact the forensic community and humanity as it suggests that additional steps are required to reduce drug abuse in teenagers.

In the United States, there is growing concern about an increase in illicit drug use and associated fatalities among young people, especially teenagers. The Office of the Chief Medical Examiner (OCME) has recorded a significant increase of drug abuse deaths among teenagers in Maryland since 1999. This study focuses on the trend and pattern of fatal drug abuse among teenagers in the State of Maryland investigated by the OCME.

A retrospective study of Maryland OCME cases, over a 16-year period between 1991 and 2006, yielded a total of 149 deaths caused by drugs of abuse among teenagers age 13 –19 years in Maryland. Ninety-six deaths (65%) were the result of narcotic drug use, such as heroin/morphine (N=59), methadone (N=18), methadone and heroin/morphine (N =3), oxycodone (N= 9), fentanyl (N = 5), tramadol (N= 1), and propoxyphene (N= 1). Twenty-nine deaths (19%) involved both narcotics and cocaine use; 4 deaths (3%) involved both narcotics and methylenedioxymethamphetamine (MDMA), and 6 deaths (4%) were due to cocaine use exclusively. Volatile substances accounted for 14 deaths (9%), including butane (N= 6), freon (N=4), nitrous oxide (N= 3), and propane (N= 1).

Over the sixteen-year study period, the number of drug abuse deaths among teenagers increased sharply in Maryland from six cases in 1991 to 15 cases in 2006, a 150% increase. Narcotic drugs, especially heroin/morphine and methadone played a major role in the rising number of teenager drug abuse deaths. From 1991 to 1998, 22 teenagers died of narcotic drug intoxication. Of the 22 narcotic drug abuse deaths, 18 deaths were due to heroin/morphine use, 1 death was from methadone and heroin/morphine use, 2 deaths involved fentanyl use, and 1 death was caused by tramadol use. From 1999 to 2006, 74 teenagers died of narcotic drug use, with 40 deaths due to heroin/morphine use, followed by methadone use (N = 18), oxycodone use (N =9), fentanyl use (N =3), methadone and heroin/morphine (N =2), and propoxyphene (N =2).

Maryland is made up of 23 counties and Baltimore City. The majority (77.9%) of teenager drug abuse deaths occurred among county residents. Teenager drug deaths in the counties increased sharply from 30 cases during 1991 through 1998 to 86 cases during 2000 through 2006. The number of teenager drug abuse deaths in Baltimore City stayed relatively constant over the 16-year period with a slight decrease (19 cases between 1991 and 1999; 16 cases between 2000 and 2006).

In Maryland, drug abuse deaths in teenagers occurred more frequently in March, October, and November. Fewer deaths occurred in April, June, and December than in the other months.

White teenagers (86%) were much more frequently involved in drug abuse deaths than black teenagers. More male teenagers (81%) died of drug abuse than female teenagers. During 2005 and 2006, 17 of 166 teen homicide victims (10%) showed evidence of some form of illicit drug activity.

Drug Abuse Deaths, Epidemiology, Forensic Toxicology

K9 Interpretation of Fentanyl in Postmortem Cases

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After attending this presentation, attendees will have an increased knowledge of the application of High Performance Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) to the analysis of Fentanyl, in postmortem specimens and of the concentrations of fentanyl encountered during analysis of these specimens.

Fentanyl is a synthetic opiate analgesic. It is frequently administered as a transdermal patch and is a substance of abuse. This presentation will impact the forensic community by increasing awareness of the very high potency of fentanyl and the frequency of its use both alone and in combination with other intoxicants. The presentation will also demonstrate the need for very sensitive analytical methodologies for its detection and quantitation.

Methods: Twenty-three medical examiner cases containing fentanyl were identified by a positive ELISA, by a gas chromatographic/mass spectrometric (GC/MS) screen, or by the presence of a transdermal patch on the body. Fentanyl was quantified in blood by LC/MS/MS using multiple reaction monitoring techniques and fentanyl-D5 as internal standard. Molecular ions (m/z 337.3 and 342.3) were refragmented to yield ions of masses 188, 132 and 105 (Fentanyl) and 188, 137 and 105 (Fentanyl-D5). Specimens, standards and controls (0.5 mL) were basified by addition of 0.05 mL concentrated ammonium hydroxide solution and were extracted with 1.25 mL hexane:ethanol (95:5). The organic solvent was decanted, forced through a 0.2 micron acrodisc syringe filter and evaporated. The residues were reconstituted into 0.25 mL mobile phase and twenty microliters (20µL) were injected. Chromatography was performed on a Varian Pursuit column (C-18, 3 micron, id = 2 mm, l = 50 mm) and an aqueous formic acid : acetonitrile gradient solvent system. The calibration range of the assay was 0.5 to 20 µg/L. Positive controls were run at 2.5 and 15 µg/L. The limit of quantitation (generally 0.5 µg/L) was defined as the lowest standard or control that assayed within twenty percent of target, with acceptable ion

qualifier ratios. Samples containing greater than 20 µg/L of fentanyl were diluted prior to analysis.

Results: Fentanyl was the major intoxicant in 13 cases and was present in a further 10 cases in combination with at least one other significant intoxicant, such as methadone, an opiate, cocaine or ethanol. The fentanyl content of the 13 fentanyl-"primary" cases ranged between 3 and 49 µg/L (14 + 12 µg/L). In the 10 cases that contained other significant intoxicants, fentanyl concentrations were between 0.5 and 18 µg/L (5 + 5 µg/L) and seven of these cases contained less than 5 µg/L (0.5 to 4.6 µg/L) of fentanyl.

Conclusion: These data indicate that fentanyl is frequently encountered in combinations with other drug substances and that, in these combinations, even very small amounts of fentanyl may contribute to lethality. Sensitive methods of analysis, such as LC/MS/MS are required for quantitation of fentanyl in these cases. The data also indicate that interpretation of fentanyl levels in postmortem cases must be done on a case-by-case basis and must consider fully the combined effects of all intoxicants present.

Fentanyl, Postmortem, LC/MS/MS

K10 DART-TOF Applications in Toxicology and Controlled Substances

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After attending this presentation, attendees will be aware of applications for Direct Analysis in Real Time – Time of Flight Mass Spectrometry (DART-TOF) in the disciplines of toxicology and controlled substances. These applications were based on observations from data collected from casework and in the creation of user libraries. These libraries are comprised of pure compounds, analytes in solution and pharmaceutical preparations.

This presentation will impact the forensic science community by providing sample data and user libraries on a relatively new instrument so that forensic laboratories can understand how this new instrumentation could assist forensic laboratories in casework production.

DART-TOF is a novel technique that allows for the quick identification of target analytes. DART is unique ionization source in that it uses an open air sampling interface for rapid sampling and that enables the TOF-MS to provide real time mass spectrometry data. The selectivity of the instrument is based upon the high degree of mass accuracy and the fragmentation observed at high voltages. The DART-AccuTOF© instrument used was created by and purchased from JEOL.

In the discipline of controlled substances, the purpose of DART is to improve the efficiency of the identification of controlled substances, thus increasing the output production of the laboratory. Case samples were analyzed to evaluate the ability of this instrument to meet this objective. Concurrently, a library of over 400 controlled and non-controlled substances was constructed and compared to calculated masses found in the literature.

The types of samples currently being evaluated are Marihuana and pharmaceutical preparations. For Marihuana, it is important to be able to distinguish Δ9-Tetrahydrocannabinol and Cannabidiol in order for this instrument to be considered a conclusive examination for this substance. These two compounds have identical molecular formulas and can not be distinguished without fragmentation. This fragmentation was observed at higher voltages and different ionization modes. Signature fragments are being evaluated to distinguish compounds from one another when possible. Compounds that are potential interferences and have the same molecular weight as Δ9-Tetrahydrocannabinol was evaluated for their signature fragments.

Pharmaceutical preparations are an ideal candidate for quick analysis due to the fact the imprinted logo presumptively identifies which compounds are present, both controlled and non-controlled. Two compounds of interest due to the frequency of submission to the laboratory are codeine and hydrocodone. These compounds have identical molecular formulas and these compounds are being evaluated to determine how they can be distinguished by their signature fragments. A tablet library was also being constructed so that tablets from specific companies could be identified not only by their primary compound, but also by the ratio of inactive and inert ingredients.

In the discipline of toxicology, DART-TOF can be used to examine gastric contents and tablets found in gastric contents. Results from the analysis of toxicological specimens analyzed by DART-TOF will be presented.

DART, Controlled Substances, Toxicology

K11 Urinary Elimination of 11-Nor-9-Carboxy-Δ^9-tetrahydrocannabinol in Cannabis Users During Continuously Monitored Abstinence

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Attendees will learn about urinary elimination of 11-nor-9-carboxy-Δ^9-tetrahydrocannnabinol (THCCOOH) from cannabis users that self-reported weekly to daily use. These urine specimen data describe parameters pertinent to cannabis elimination.

Four inpatient studies of cannabis users (N=60; 6,158 individual urine specimens) were performed at the NIDA IRP with the objectives of examining the time course of THCCOOH elimination in urine. Protocols were approved by the NIDA IRB and each volunteer gave written informed consent. Subjects resided on a closed research unit under continuous medical surveillance. Individual urine specimens were collected *ad libitum* for up to 30 days. Volunteers consisted of 50 African Americans, 5 Caucasians, 3 Hispanics, 1 mixed race, and 1 American Indian. There were 46 male and 14 female participants ages 20 to 42 years. All self-reported cannabis dependence or use, and had a positive urine cannabinoid specimen to support exposure.

Specimens were screened by immunoassay with values ≥50 ng/mL classified as positive for cannabinoids. Urine specimens were confirmed for THCCOOH by gas chromatography/mass spectrometry (GC/MS) following base hydrolysis and liquid-liquid extraction. The limit of quantification was 2.5 ng/mL. Cannabinoid GC/MS concentrations (ng/mL) were normalized to the urine creatinine concentration (mg/dL) to account for the state of hydration and reduce variability with the final normalized units expressed as ng/mg. In 60%, the maximum normalized concentration occurred in the first urine specimen. In the other 40%, peak THCCOOH concentrations occurred as long as 2.9 days after admittance.

Data were divided into three groups, 0 - 50, 51 - 150, and >150 ng/mg, based on the normalized urine THCCOOH concentration in the first specimen after admittance. Mean ± SD, median and range of concentrations in the 0 - 50 ng/mg group (N = 19 subjects) were 23.7 ± 15.9, 27.8, 0 – 47.3 ng/mg. Data for the other groups were 97.2 ± 22.6, 96.1, 61.9 – 142.2 ng/mg

and 339.8 ± 247.3, 283.0, 155.1 – 1165.9 ng/mg, respectively for the 51 - 150 (N = 21 subjects) and >150 ng/mg (N = 20 subjects) groups. The mean intervals until the first negative specimen were 0.6, 3.2, and 4.7 days, respectively for the three groups. Mean % detection rates (percentage of positive specimens divided by total urine specimens for the day) on the day of the first negative specimen were 57.6, 73.4, and 79.8%, respectively. Mean times for the last positive urine specimen were 4.3, 9.7, and 15.4 days, respectively. The maximum time until the last positive urine specimen was 21.8 days for the 0 -50 ng/mg group, 25.3 days for the 51 – 150 ng/mg groups and 29.8 days for the >150 ng/mg group. These data reflect that the greater the initial THCCOOH concentration, the greater the interval until the first negative and last positive specimen.

These data will impact the field of forensic toxicology by increasing our understanding of cannabinoid elimination and improving interpretation of cannabinoid urine tests.

Cannabis Users, THCCOOH, Urine

K12 The Role of Steroid Abuse in Violent Deaths: A Case Report

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After attending this presentation, attendees will better understand the possible role of steroid substances abuse in violent deaths.

This presentation will impact the forensic community by providing additional support that extreme violence, homicide and suicide can be associated with steroid abuse.

A review of the literature revealed the association between substance abuse and criminal behavior. Anabolic androgenic steroids (AAS) are widely used by athletes to help increase strength and muscle mass. However, these substances can affect central nervous system causing irritability, anger and agitation and other psychiatric symptoms.

Methandriol (17α-methyl-5β-androstan-3α,17β-diol) is a synthetic anabolic steroid that is administered orally or intramuscular to treat androgen deficiency, rare forms of aplastic anemia or to counteract catabolic states, for example after major trauma. Methandriol is also commonly used by body builders to cause an increase of muscle bulk. Together with cardiovascular, endocrine, gastrointestinal and hepatic collateral effects, psychiatric changes can occur during prolonged use or after cessation of this agent. Mania and psychotic symptoms of hallucination, delusion and depression are described in AAS abusers. There is a considerable debate concerning effects of synthetic derivatives of testosterone on aggressive and on criminal behavior with domestic violence, suicide, and homicide.

This report documents a case of homicide-suicide committed by a law enforcement officer with no apparent reason. The perpetrator was a 29-year-old man; he had been employed with police service for nearly ten years. In a recent physician's assessment no psychiatric disorder or illicit drug and alcohol use were reported. He had no criminal record or reported previous instances of violent behavior. He was happily in love with a girl who described him as a mild-mannered, kindly and caring, although he did exhibit mild episodes of depression and anxiety.

He was pursuing body-building activities five year prior to the incident, and his relatives suspected he occasionally used steroids during the last six months prior to the fatal event. While he was working in the police station it is reported that he argued with his police lieutenant for trivial reasons. His colleagues reported his temper shortened incredibly in the following hours and, during the night, he took his service gun and fired against an officer who was sleeping in the same police station, killing him with six gunshot wounds to the head, neck, thorax, and upper extremities. He subsequently killed himself with a gunshot wound to the head.

At autopsy the pathologist observed his height was 190 cm and weight 90 Kg with diffuse and harmonic muscles hypertrophy. A left temporal entrance gunshot wound with central skin defect of 1.4 cm diameter and no adaptable ragged margins was disclosed. Another gunshot wound was ascertained at the right auricle. No more injuries or diseases were found except for mild myocardial hypertrophy and liver steatosis, as correspondent microscopic findings confirmed later. Toxicological examination of post-mortem blood and urine samples were conducted to determine whether death was related to illicit substance's abuse. The analytical procedures consisted of immunoassays and gas chromatographic methods, utilizing mass spectrometry detection (GC-MS).

A concentration of 3.4 ng/ml of Methandriol was detected in femoral blood as well as metabolites in urine with a concentration of 5.2 ng/ml. Blood alcohol concentration was determined to be 0.4 g/L and no alcohol was detected in the urine.

Although is not possible to exclude unknown problems with the victim, and his relatives or working history, autopsy findings and officer's toxicological analysis performed with GC-MS suggests that Methandriol abuse represented the principal etiology of the officer's violence, culminated in this tragic episode of homicide-suicide. This case confirms the possible role of steroid abuse in violent behavior.

Methandriol, Steroid Abuse, Homicide

K13 Specificity Characteristics of Buprenorphine Immunoassays and Their Effects on the Correlation of Immunoassay Apparent Analyte Concentration With GC-MS Concentrations of Buprenorphine and Its Metabolites in Urine

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After attending this presentation, attendees will better comprehend the effect of immunoassay (IA) specificity on the commonly adapted 2-stage test methodology - IA and GC-MS for preliminary and confirmatory tests - for the analysis of buprenorphine (B) and its metabolites in urine specimens. This presentation will impact the forensic science community by reporting: (a) specificity characteristics of various commercially-available B IAs, and (b) the effect of these characteristics on the correlation of IA apparent B concentration in clinical urine specimens to the concentrations of B and its metabolites (norbuprenorphine, NB; B glucuronide, BG, and NBG) as determined by GC-MS.

Performance characteristics of five B ELISA (Immunalysis, Neogen, Diagnostix, IDS-B, IDS-NB) and one analyzer-based (CEDIA by Microgenics) reagents currently available from commercial sources were studied to better understand their analytical parameters, including calibration, cross-reacting characteristics, assay precisions and others. Information thereby derived were applied to the analysis of clinical urine specimens collected from heroin addicts under B "treatment" following required IRB protocols. Resulting IA *apparent* analyte concentrations were correlated against the concentrations of various metabolites as determined by GC-MS to better understand the effects of these IAs' specificity characteristics.

ELISA reagents studied were found to exhibit significant cross-reactivity toward BG in the order shown below: IDS-NB > Neogen > Diagnostic > IDS-B, while Immunalysis and Diagnostix reagents were found to significantly cross-react with NB. IDS-NB and Diagnostix reagents were also found to exhibit significant cross-reactivity toward NBG. The analyzer-based CEDIA reagent was found to show significant cross-reactivity toward

BG and some cross-reactivity toward NBG and NB. Unlike other reagents studied, IDS-NB reagent was also found to exhibit significant cross-reactivity toward morphine, codeine, hydrocodone, hydromorphone, oxycodone, and naloxone.

With different cross-reactivity characteristics, apparent analyte concentrations derived from various IAs were found to correlate with the metabolites' concentrations (as determined by GC-MS) in different ways. For example, showing significant cross-reactivity toward BG, CEDIA reagent generated apparent B concentrations that do not correlate well with the concentrations of B (Figure 1A) or BG (Figure 1B) alone, but with significant correlation with the total concentration of B (Figure 1C).

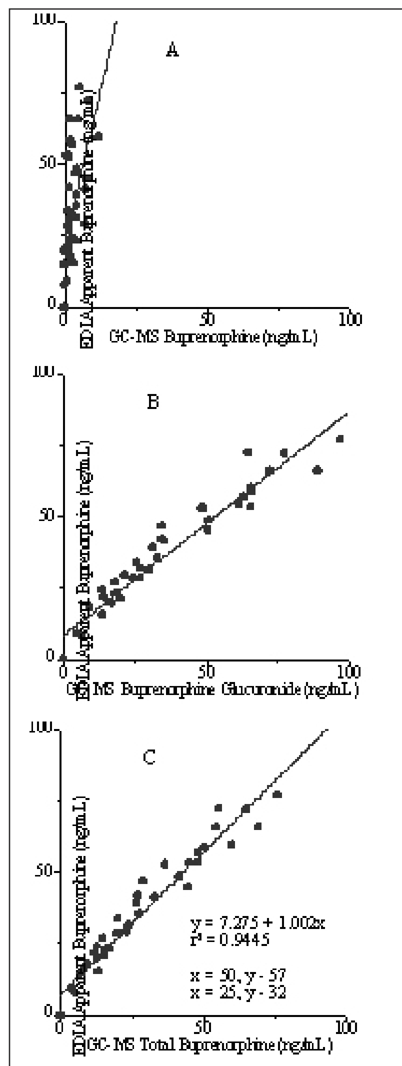

Figure 1. Correlation of CEDIA apparent buprenorphine concentration against GC-MS buprenorphine (A), buprenorphine glucuronide (B), and total buprenorphine (C) concentrations.

Buprenorphine, Glucuronide, Immunoassay

K14 Analysis of Goldenseal, *Hydrastis canadensis L.*, and Related Alkaloids in Urine Using HPLC With UV Detection

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After attending this presentation, attendees will be able to better detect alkaloids from Goldenseal, *Hydrastis canadensis L.*, as adulterants in urine samples using high performance liquid chromatography.

This presentation will impact the forensic science community by serving as a key aspect in detecting isoquinoline alkaloids resulting from Goldenseal, *Hydrastis canadensis L.*, in a basic urine drug test, which may lead to eliminating false negative drug results in toxicology laboratories.

Goldenseal root powder, *Hydrastis canadensis L. (family Ranulanceae)*, is one of the top selling herbal supplements on the market in the United States today. This may be in part to Goldenseal's use as a detoxing agent that drug users believe may provide false negative results during a urine drug test. This indigenous North American perennial herb is widely cultivated, and its extracts have been used for a variety of medicinal purposes and also as a dye. *Hydrastis canadensis L.* has been reported to contain several isoquinoline alkaloids, including 2-4% hydrastine and 2-3% berberine by weight. A number of other alkaloid containing plants have been reported for use in masking urine drug tests instead of Goldenseal, including Chinese Goldenthread (*Coptis chinensis*), yellow root (*Xanthorhiza simplicissima*), and Oregon grape (*Mahonia aquifolium*).

The main objective of the project was to create a test method for toxicology laboratories to detect Goldenseal, and related alkaloids, in urine samples using HPLC. An isocratic HPLC method with UV detection was developed to extract the alkaloids from 5 mL of urine. The urine samples were spiked with 100 µL of alkaloid standard (containing different concentrations of berberine and hydrastine). 5 mL of a 3:1 chloroform:isopropanol ($CHCl_3$:IPA) extraction solvent was agitated with the 5 mL of urine sample and the $CHCl_3$:IPA layer was removed. This process was repeated a second time with the $CHCl_3$:IPA solutions combined and concentrated using a stream of nitrogen gas. The residue was then reconstituted with 100 µL mobile phase and 10 µL injected onto the HPLC column. A mobile phase was prepared of 320 mL acetonitrile and 680 mL mobile phase buffer (1000 mL HPLC grade water, 2.3 g ammonium acetate, and 2 mL triethylamine). A 17 minute isocratic method was developed, with a flow rate of 2.0 mL/min, and UV detection at 230 nm using a C18 (250 mm X 4.6 mm) column at room temperature. The method showed good linearity with spiked urine samples for berberine and hydrastine standards at a range of approximately 12.74 ng/mL to 12.52 µg/mL. LOD for berberine in urine was 12.74 ng/mL and the LOD for hydrastine in urine was 54.5 ng/mL. Urine samples were also spiked with Goldenseal powder and liquid to determine whether Goldenseal would also show a presence in urine samples. The results show this method will enable laboratories to test for the herbal supplement in submitted urine samples on an as needed basis to further test suspect adulterated urines. The method used for the detection of goldenseal is not recommended however for use as a screening procedure in a production laboratory without the use of an autosampler.

Goldenseal, High Performance Liquid Chromatography, Toxicology

K15 Performance Characteristics of Cozart Rapidscan® Oral Fluid Drug Testing Following Controlled Dental Anaesthetics Infiltration

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After attending this presentation, attendees will understand some aspects of oral fluid drug testing after controlled dental anaesthetics infiltration and its interferences with the final results.

These early findings will impact the forensic community by providing a starting point for effectiveness of oral fluid drug testing when performed on patients who have undergone dental treatments.

Oral fluid is an interesting alternative matrix for drug testing in many environments, including law enforcement, workplace drug testing, and drug treatment facilities. The ease with which specimens can be collected and the potential for oral fluid drug concentrations to reflect blood-drug concentrations make it potentially valuable in a forensic setting. The possible effects on drug detection and quantification in patients who received local anaesthetics for dental treatments have not been examined. Drugs generally appear in oral fluid by passive diffusion from blood, but also may be deposited in the oral cavity during oral administration. Anaesthetic metabolites can be detected in oral fluid and could mimic drug metabolites thus giving a distorted result. The purpose of this study was to determine the performance characteristics of the Cozart Rapidscan oral fluid drug testing for the detection of cocaine and cocaine metabolites in oral fluid following controlled infiltration of Mepivacaine, Lidocaine, and Articaine.

Three different local dental anaesthetics were employed for this research: Mepivacaine 2% (Carboplyna, Dentsply Italia), Mepicavaine 3% (Scandonest, Ogna spa Italia), Lidocaine 2% (Ecocain, Molteni Dental srl Italia) and Articaine 4% (Alfacaina SP, Spada Dentsply Italia). Five volunteers, provided with informed consent, were selected and received local anaesthetic infiltration bilaterally in vestibular fundus of the mental area of the mouth, in different settings: 1.8 ml and 3.6 ml of mepivacaine 2% with 1:100.000 adrenaline; 1.8 ml and 3.6 ml of mepivacaine 3%; 1.8 ml and 3.6 ml of lidocaine 2% with 1:50.000 adrenaline; 1.8 and 3.6 ml of articaine 4% with 1:100.000 adrenaline. The four selected anaesthetic molecules were tested at cutoff concentrations. Oral fluid specimens (N = 200) were taken before anaesthetic infiltration and after 30, 60, 120 and 240 minutes following the infiltration, and were analyzed for cocaine and cocaine metabolites using Cozart rapidscan.

It was concluded that articaine local anaesthetic has a positive interference with the effectiveness of the saliva test for cocaine and cocaine metabolites with the Cozart device at a cutoff of 5 microgram/mL, while mepivacaine and lidocaine have no interference. Results from a larger group of subjects would be needed in order to validate these findings.

Oral Fluid, Cocaine, Dental Anaesthetic

K16 Evaluating the Significance of Variation of Drug Concentrations in Antemortem and Postmortem Blood and Tissue Samples

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Upon viewing this presentation, attendees will be able to examine the postmortem changes in blood drug concentrations over time. This will aid them in determining the role such factors as postmortem interval, storage conditions and biochemical transformations have on the interpretive value of quantitative data.

This is the first known comprehensive study of its kind in humans where samples are analyzed starting from the antemortem phase and followed through the postmortem phase. This presentation will impact the forensic science community by providing information that will aid in the process of relating antemortem to postmortem drug concentrations.

Postmortem (PM) forensic toxicology seeks to determine what role, if any, drugs or poisons played in causing or contributing to death. Drug related deaths can encompass everything from overdoses to non-compliance with prescription medications to drug-drug interactions. In situations where chemical substances may have played a role in a death, it is necessary to first identify which drug(s) are present, and then to determine the concentration(s) in blood and, sometimes, in tissues from the deceased. Proper scientific interpretation of postmortem drug concentrations may be critical in the correct assessment of the cause of death. To date there is no definitive way to correlate PM toxicological results with antemortem (AM) drug concentrations because the accurate relationship between PM drug concentrations to perimortem concentrations has yet to be established. The main reason for the hindrance is a phenomenon known as postmortem redistribution (PMR).

Put quite simply PMR is the name given for the movement of chemicals and drugs within the body after death. While living processes (including absorption, distribution, metabolism and excretion) have ceased, decomposition processes have begun. It is in-part, the decomposition processes (including autolysis), which allow for the release of drugs from their stored depots in tissues. The extent of redistribution may vary based upon a number of factors including: postmortem interval, sampling site (i.e. peripheral vs. central), environmental factors (i.e., temperature, humidity, etc.) and subsequent microbial activity present as a result of the aforementioned. Additionally, the chemical properties of drugs (and drug classes) play a significant role in their ability to redistribute. Some drug classes have a higher propensity than others to be sequestered in living tissues and subsequently released and redistributed after death.

This two part comprehensive study first examined the differences in AM and PM drug concentrations in blood and serum. AM samples collected from area hospitals were analyzed with the related PM samples obtained from the Miami-Dade County Medical Examiner's Office located in Miami, Fl. Multiple cases were collected, analyzed and evaluated both individually and collectively. Secondly, a study to establish of the stability of drugs within the preserved PM samples was conducted. The PM samples were analyzed at multiple time points to determine the pattern of drug concentration changes. The results of each individual case were evaluated, and the cumulative data were examined for evidence of trends or patterns.

All of the samples were maintained and analyzed solely on the grounds of the Miami-Dade County Medical Examiner's Office. The majority of the samples were stored in a walk-in refrigerator (7ºC), while some additional PM samples were frozen (-85º) upon receipt. Both liquid-liquid and solid-phase extraction techniques were utilized to produce specimens analyzed on such instruments as: GC/MS, GC/NPD and GC/ECD.

To date, PM concentrations of alprazolam, diphenhydramine, and methadone all show a decline of ≥ 20% over a two month period. When the same drugs are used to examine the differences between AM to PM drug

concentrations, almost invariably the AM concentrations are significantly (≥ 50%) lower than the PM.

The data generated here will help establish a correlation between PM and AM drug concentrations. In the future this information will help guide PM interpretation and give enhanced credibility to the field of PM toxicology as a whole.

Toxicology, Postmortem Redistribution, Postmortem Release

K17 A Two-Dimensional-Cryofocusing GC/EI-MS Method for Determination of Δ^9-tetrahydrocannabinol, 11-Hydroxy-Δ^9-tetrahydrocannabinol, and 11-Nor-9-carboxy-Δ^9-tetrahydrocannabinol in Human Urine

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The objective of this presentation is to provide a detailed description of a GC/MS procedure for the simultaneous quantification of THC, 11-OH-THC, and THCCOOH in human urine. The method utilizes two-dimensional chromatography and cryofocusing to enhance resolution and improve sensitivity.

The presentation will impact the forensic science community by allowing participants to develop and apply two-dimensional chromatography to the quantification of THC and major metabolites. The method may be a useful analytical procedure in forensic toxicology applications.

A sensitive and specific two-dimensional (2D) gas chromatography/electron impact-mass spectrometry (GC/EI-MS) method for simultaneous quantification of Δ^9-tetrahydrocannabinol (THC), 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC), and 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THCCOOH) in human urine was developed and validated. The method employs 2D capillary GC and cryofocusing for enhanced resolution and sensitivity. GC separation of trimethylsilyl derivatives of analytes was accomplished with two capillary columns in series coupled via a pneumatic Deans switch system. Detection and quantification were accomplished with a bench-top single quadrupole mass spectrometer operated in electron impact-selected ion monitoring mode.

To ensure complete hydrolysis of conjugates and capture of total analyte content, urine specimens were hydrolyzed by two methods in series. Two mL urine fortified with THC-d_3, 11-OH-THC-d_3, and THCCOOH-d_3 was hydrolyzed with 5000 units/mL of *Escherichia coli* b-glucuronidase (pH 6.8) for 16 h at 37°C in a shaking water bath followed by a second hydrolysis utilizing 10N NaOH at 60°C for 20 min. Specimens were adjusted to pH 5-6.5 with concentrated glacial acetic acid. Two mL of acetonitrile were added to precipitate protein followed by 2 mL 2N sodium acetate buffer (pH 4.0). Specimens were centrifuged and supernatants applied to conditioned solid phase extraction (SPE) columns. SPE columns (Clean Screen ZSTHC020, United Chemical Technologies) were washed with 3 mL deionized water, 2 mL 0.1N hydrochloric acid /acetonitrile (70:30 v/v), and dried by full vacuum for 10 min. After priming the sorbent bed with 0.2 mL hexane, analytes were eluted with 5 mL elution solvent (hexane:ethyl acetate 80:20 v/v) into tubes containing 0.5 mL ethanol and dried under nitrogen. Extracts were reconstituted with 25 µL acetonitrile, transferred to autosampler vials, and 20 µL BSTFA was added. Vials were capped and derivatized at 85°C for 30 min.

2D chromatographic separation was achieved with a primary DB-1MS capillary column (15 m x 0.25 mm i.d., 0.25 µm film; Agilent Technologies) and a secondary ZB-50 capillary column (30 m x 0.32 mm i.d., 0.25 µm film; Phenomenex). One µL derivatized extract was introduced in splitless injection mode. The Deans switch valve was programmed to divert "cuts" of the analyte elution bands to the secondary GC column for further chromatographic resolution. The secondary column was inserted through the cryogenic trap and the effluent end interfaced to the MSD for detection and quantification. Three analytes were quantified simultaneously with 2.5 to 300 ng/mL dynamic ranges for THC and THCCOOH and 2.5 – 150 ng/mL for 11-OH-THC. Calibration curves exhibited coefficients of determination (r^2) of 0.99 or greater (n = 12). Accuracy ranged from 87.6% to 102.1% for all analytes. Intra- and inter-assay precision, as percent relative standard deviation, were less than 8.6% for all analytes. Extraction efficiencies were 34.6 – 38.9% for THC, 44.0 – 52.8% for 11-OH-THC, and 39.3 – 54.9% for THCCOOH.

The combination of 2D-GC and cryogenic focusing achieved improved resolution of analyte from complex matrix components. The result was a rugged, flexible method with enhanced resolution power and lower detection and quantification limits compared to single dimensional chromatography. Focusing of the analyte band at the head of the secondary column markedly enhanced the chromatographic signal-to-noise (S/N), improving sensitivity. The method employs a rapid SPE and utilizes readily available single quadrupole GC/MS instrumentation. Acceptable assay characteristics and enhanced analytical sensitivity with improved S/N and detection limits were achieved. This method was applied to the analysis of urine specimens collected from individuals participating in controlled cannabis administration and monitored withdrawal studies, and may be a useful analytical procedure in forensic toxicology applications.

GC/MS, THC, Two-Dimensional Chromatography

K18 Simultaneous LC-MS/MS Quantification of Opiate, Cocaine, and Metabolites in Urine of Pregnant Substance-Abuse Treatment Participants

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After this presentation, attendees will be knowledgeable about opiate, cocaine and metabolites concentrations in human urine after illicit opioid and cocaine use by pregnant women.

The simultaneous LC/MS/MS analysis of 26 opiate and cocaine analytes in urine demonstrated that this technology was useful for monitoring multiple biomarkers of illicit drug use in opiate and cocaine dependent pregnant women. This presentation will impact the forensic science community by presenting data that will be evaluated to determine potential correlations with opioid and cocaine concentrations in meconium from infants of the women and with neonatal outcome measures.

Methadone maintenance is the only currently recognized pharmacotherapy for opiate dependency during pregnancy. Urine testing is an integral component of drug treatment, is a deterrent to drug use and is the most objective measure of drug use and effectiveness of new drug treatments. Urine drug testing provides a long detection window for drug abuse, from several days for opiates and cocaine, up to a month for chronic cannabinoid use.

Fifteen pregnant heroin dependent women from the Center for Addiction and Pregnancy (CAP) at the Johns Hopkins Bayview Medical Center (JHBMC) participated while enrolled in methadone maintenance treatment. Eleven African American and four Caucasian first-time drug treatment seekers had a mean ± SD age of 29.5 ± 6.7 years (range 19-40 years) and were between 8 and 28 weeks of gestation. Throughout gestation, participants received daily methadone (mean dose 75 ± 17 mg/day; range

45-110 mg/day), weekly individual and group counseling and specialized prenatal care. The protocol was approved by the JHBMC and the National Institute on Drug Abuse Institutional Review Boards. Participants provided written informed consent and earned vouchers for negative urine tests as part of behavioral contingency management.

Participants joined the study as early as eight weeks estimated gestational age (mean number of weeks on study 17.0 ± 5.8; range 8.2-27.2 weeks) and visited the clinic seven days per week. A variety of biological specimens, including urine, oral fluids, sweat and hair, were collected at fixed times during the study, under direct observation by trained staff. Urine samples were collected three times a week and stored at -20°C until analysis. The number of specimens collected was dependent on the enrollment period. A total of 284 urine specimens were collected from fifteen participants with a mean pH of 6.7 ± 0.8 (range 4.3-8.8). LC-APCI-MS/MS analyses were performed using an LCQ Deca XP ion trap mass spectrometer, equipped with an orthogonal APCI source, and interfaced to a Surveyor HPLC system. 100 µL of urine was fortified with deuterated internal standard working solution, briefly vortex-mixed and centrifuged to remove large particles (5 min at 510 *g*). Ten µL of supernatant were injected onto the LC-MS/MS. Pre-concentration during sample preparation was not required based on the sensitivity achieved. Urine specimens were analyzed for heroin and metabolites (morphine, normorphine, 6-acetylmorphine, codeine, acetylcodeine, norcodeine, noscapine and papaverine) and concurrently for cocaine and metabolites [ecgonine, ecgonine methyl ester (EME), ecgonine ethyl ester (EEE), anhydroecgonine methyl ester (AEME), *p*-hydroxybenzoylecgonine (*p*-OHBE), *m*-hydroxybenzoylecgonine (*m*-OHBE), benzoylecgonine (BE), benzoylnorecgonine (BNE), *p*-hydroxycocaine (*p*-OHCOC), *m*-hydroxycocaine (*m*-OHCOC), and norcocaine based on selected reaction monitoring.

Opiates were detected in 149 (52.5%) of urine specimens. Of fifteen participants, one had no opiate positive results, five had less than 20%, eight between 20% and 90%, and two more than 90% positive tests for opiates, often with high concentrations of morphine-3-glucuronide. Morphine, normorphine, 6-acetylmorphine (6-AM), codeine and norcodeine were the other primary opiates identified in urine specimens. 30 % of opioid positive specimens contained 6-acetylmorphine, a specific biomarker of heroin use. 165 (58%) specimens from all 15 participants tested positive for one or more cocaine analytes. Seven subjects had between 20 and 50% cocaine positive specimens, 5 between 50 and 80%, 3 more than 80%, and cocaine was found in all urine specimens of one subject, often with high concentrations. EME and BE were the primary cocaine analytes in urine specimens. EME was detected in 123 (43.3%) urine specimens with a median concentration of 115 ng/mL (range 51-33002 ng/mL), and BE in 84 (29.6%) with a median concentration of 47 (10-73758).

Opiate, Cocaine, LC-MS/MS

K19 Detection of Alcohol Metabolites in Urine Using HPLC With Conductivity Detection

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The goal of this presentation is to expand the current knowledge of detection methods for three Phase II ethanol metabolites (ethyl glucuronide, ethyl sulfate, and ethyl phosphate), and offer a simple, comparable method.

This presentation will impact the forensic science community by allowing the detection of alcohol intake without the worry of contamination due to bacteria, as well as offering a confirmatory analysis mid-assay.

After consumption of alcohol, the bulk of the ethanol dose (95-98%) is eliminated in a two stage oxidation in the liver, first to acetaldehyde then further to acetic acid. A very small fraction of ethanol (<0.1%) undergo phase II conjugation reactions to produce ethyl glucuronides via UDP-glucuronidase, ethyl sulfates via sulfotransferases, and ethyl phosphate via dephosphorylation of ATP. Modern postmortem and behavioral toxicology has focused on glucuronides, allowing for easy detection due to high abundance of metabolites from that pathway. This detection is not without its problems, including false negatives due to bacterial infections.

Previous work in this laboratory has used pulsed amperometric detection to detect ethyl-glucuronide, a metabolite of alcohol. This work expands on that, by allowing all three metabolites, ethyl glucuronide, ethyl sulfate, and ethyl phosphate to be detected in a single chromatographic run. All three are ionic in biological matrices, including urine, making them ideal candidates for conductivity detection following ion chromatographic separation.

This poster will outline the development of the ion chromatographic separation of the three metabolites and their subsequent detection using conductivity detection. Analytical figures of merit will be given, and the method will be compared against existing approaches. Sample preparation will be discussed in detail. This project will have long standing effects in the forensic science community by allowing detection of alcohol intake without the worry of contamination due to bacteria, as well as offering a confirmatory analysis mid-assay.

Alcohol, HPLC, Conductivity

K20 Extraction of Heroin From *Lucilla Sericata* Larvae by Pressurized Fluid Extraction

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The aim of this presentation is to outline a method for the rapid extraction of heroin and its metabolites from maggots using pressurized fluid extraction. It will focus on the preparation and extraction of samples and compare analysis times and efficiency with established extraction methods.

This presentation will impact the forensic science community by bringing attention to the potential use of pressurized fluid extraction for applications in entomotoxicology. A rapid and efficient extraction of heroin and its metabolites from a larval matrix is presented. This method could be extended to similar cartilage-like matrices such as finger and toenails.

The use of fly larvae as toxicological specimens was first reported in 1980 and has since been widely studied and utilized forensically as a means of diagnosing death by drug intoxication. Fly larvae (Diptera) are frequently found on decomposing bodies long after tissue samples (blood, urine, organs) commonly used for toxicological analysis are no longer available or suitable for analysis. Diptera, may feed on the tissue of a deceased individual who had taken drugs while alive, thereby ingesting any remaining drug as well as its metabolites. Drug accumulation within the maggot occurs as it develops and analysis can provide evidence for the presence of a drug in the cadaver.

Traditional methods for the extraction of substances from maggots, including manual homogenization and sonication, can be lengthy and time consuming. In this presentation, the use of pressurized fluid extraction for the detection of heroin and its metabolites in blow fly larvae, *Lucilla Sericata* (Diptera: Calliphoridae) is offered as a rapid and simple extraction method reducing overall analysis time. *Lucilla Sericata* were reared on pork liver spiked with varying concentrations of heroin and its metabolites. Concentrations were chosen based on those commonly found in tissue from heroin overdose victims. A surrogate spike (codeine-d3) was added to track extraction efficiency. Larvae were reared at 21.2ºC with cyclical artificial lighting simulating 14h daylight and 10h darkness. Larvae were harvested at 5 days and sacrificed by freezing to -80ºC. Prior to extraction, frozen larvae were ground using a mortar and pestle in liquid nitrogen. Extraction was carried out using pressurized solvent extraction by modifying previously reported methods for the extraction of substances from tissue samples. The extraction was carried out at 100ºC and 1500 psi using methanol as the extraction solvent. The extract was evaporated to required volume with

nitrogen.

Qualitative analysis was carried out via an established and previously validated method on a gas chromatograph coupled with a mass selective detector. The instrument was operated in split mode with a 1 ul injection. An internal standard (heroin d-6) was used for the analysis.

Entomotoxicology, Pressurized Fluid Extraction, Forensic Entomology

K21 Assay of GHB Oxidation Activity Using a Succinic Semialdehyde-Hydrazine Adduct

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After attending this presentation, this presentation will provide attendees the opportunity to learn about the development of an assay for GHB oxidation, relying on the formation of a succinyl semialdehyde-hydrazine adduct, as determined by ultraviolet/visible absorbance (UV), high pressure liquid chromatography (HPLC) with UV and mass spectrometric detection (MS).

This presentation will impact the forensic and toxicological communities by introducing a new assay for the oxidation of GHB.

Forensic toxicologists and pathologists are regularly called on to evaluate the role and magnitude of effects of GHB in Drug-Facilitated Sexual Assaults (DFSA), accidental overdoses, and homicides. This laboratory is interested in the kinetics of GHB catabolism. The first step of GHB metabolism is oxidation to succinyl semialdehyde. Subsequently, oxidation of succinyl semialdehyde to succinate rapidly follows. To allow for an effective analysis of GHB metabolism based on product formation, or cofactor reduction, the assay methodology must effectively eliminate the oxidation of succinyl semialdehyde to succinate. The feasibility of utilization of hydrazine sulfate as an "aldehyde trap," allowing the termination of the reaction at succinyl semialdehyde via formation of a readily detectable, unique product, the succinyl semialdehyde-hydrazine adduct has been investigated. Previous studies suggest that hydrazine sulfate may be an effective means of trapping succinyl semialdehyde that would not be expected to interfere with the metabolism of GHB to succinyl semialdehyde.

The purpose of this study is to identify a succinic semialdehyde-hydrazine adduct for use in an HPLC-MS assay for oxidation of GHB. An HPLC method has been previously developed for identification and quantitation of succinic semialdehyde in urine. The HPLC is operated at a flow rate of 1 mL/min with a mobile phase of 80 mg/L ammonium acetate buffer (pH 3.6) in 1:1 acetonitrile:water. Post column, the flow is split 1:4 producing a flow rate of 200 µL/min to the mass spectrometer. Electrospray ionization was used in the negative ion mode. The temperature of the electrospray was set at 400ºC (Struys EA et al, J Inherit Metab Dis, 28:913, 2005).

The succinyl semialdehyde-hydrazine adducts can take several forms. The following ions have been identified when excess succinyl semialdehyde is reacted with hydrazine sulfate: succinyl semialdehyde (m/z 102) and succinyl semialdehyde-hydrazine adducts (m/z 141, 183, 225, 257). The m/z 141 is consistent with an adduct comprising a heterocyclic ring using one succinyl semialdehyde molecule and hydrazine. Higher m/z values are expected to correlate with an adduct comprising two succinyl semialdehyde molecules with hydrazine. Increasing the amount of hydrazine used in the assay causes the heterocyclic product (m/z 141) to be favored; which offers a single product measurable by UV and MS detection.

Formation of adduct is reliant upon formation of aldehyde. Therefore, quantitation of the succinic semialdhyde-hydrazine adduct allows for determination of the rate of GHB oxidation to succinyl semialdehyde. By optimizing the HPLC-MS method for the detection of the oxidized product (in this case the succinyl semialdehyde-hydrazine adducts), the method is functional for determination of the kinetics of GHB oxidation.

Gamma-Hydroxybutyrate, Assay, Succinyl Semialdehyde

K22 Cocaine Testing of Drug Treatment Patients - Comparison of Urine, Sweat, Oral Fluid, Skin Wipes, and Hair

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After this presentation, attendees will understand some benefits and limitations of urine, sweat, oral fluid, skin wipes, and hair as forensic drug testing matrices; some characteristic patterns observed in daily urinalysis; an improved sweat collection procedure; examples of false positives; and recommendations for prudent interpretations of drug test results.

This presentation will impact the forensic community by introducing ways to improve the reliability of drug testing and its interpretation/reporting.

This study compared the matrices of urine, skin swabs, sweat, and hair for monitoring cocaine use, with urinalysis being the gold standard, or arbitrator of use. Possible environmental contamination was measured through skin swabs. Unique aspects included *daily urine monitoring* (Monday-Saturday) of 35 participants for up to four weeks, simultaneous monitoring methods of all matrices, CI-GC/MS analysis to the limit of detection (LOD), and the potential for ongoing illicit drug use by participants in cocaine dependence treatment. This report expands an earlier pilot study. In the current cohort of participants, twenty showed virtually no cocaine use by urinalysis, four tested positive continuously, and eleven displayed infrequent use. Proposed new cut-off levels for the various matrices are based on receiver operating characteristic (ROC) curves widely used in clinical chemistry for cost/benefit analyses for a matrix assay using false positive and false negative rates to determine statistically how well it correctly identifies a positive result (defined as sensitivity) and how well it identifies a negative result (defined as specificity).

Identification of cocaine use was based on the intensity and shape of the urine BE excretion curve over several days and acceptable creatinine levels. Urine specimens exhibited a sensitivity of 0.86 at 100 ng/mL BE (n=934) and a specificity of 0.99. At 300 ng/mL BE, the sensitivity was 0.76 and the specificity was 0.998. The few false positives in urine were attributed to inadvertent ingestion of trace amounts of cocaine by the participants.

Skin swabs showed contamination on either hands or forehead, even with urine-negative participants. Generally, the skin contamination paralleled the drug use pattern detected by urinalysis. The amounts of cocaine on skin in some cases far exceeded the amounts attributable to drug use alone, with the excess caused by skin contact with drug residues.

Sweat was collected using PharmChek™ sweat patches applied on alternating arms at approximately four-day intervals. False positives (defined as the presence of drug or metabolite without intentional drug use) occurred at a 1.8% rate at the proposed cutoff concentrations, 75 ng cocaine/patch in this research. Using the ROC curves, the diagnostic test sensitivity was 0.60 (n=301). At the SAMHSA cutoff of 25 ng cocaine/patch a 10.6% false positive rate was observed. Pretreatment of selected patches with glycerol resulted in enhanced drug transfer from the skin to the patch when compared with non-treated patches.

Oral fluid was collected with Sarstedt Salivettes™. Oral fluid cocaine levels generally paralleled urine BE at substantially lower concentrations. For oral fluid cocaine or BE levels >15 ng/mL of extract, the diagnostic test sensitivity was 0.68 among chronic users (n=103) and 0.34 for occasional users (n=243). The specificity was 1.00 for chronic users and 0.97 among occasional users.

Hair was collected at the beginning and end of the 4-week study period. Although prior use patterns were unknown, the median cocaine concentration for African-American hair at the beginning of the study was 6.1 ng/mg hair vs. 1.2 ng/mg of Caucasian hair. African-American hair tended to retain

cocaine longer so that at the end of the study the median level was 4.9 ng/mg compared to 0.24 ng/mg for Caucasian hair. The concentrations of cocaine in the hair of individuals who were abstinent during the study period did not fall to zero even though no drug use was identified. ROC curves were not generated for hair due to limitations of the sampling interval and related hair growth.

These results indicate that, while each drug testing method has its strengths and limitations, urine appears to be less susceptible to environmental contamination than other matrices.

Cocaine, Drug Testing, Contamination

K23 Ethanol Elimination Rates From Time Discrete Blood Draws in Impaired Driving Cases

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After attending this presentation, attendees will gain insight into the pharmacokinetics of ethanol, specifically, its elimination in men. In this study, apparent elimination rates were calculated from 173 cases involving two time-discrete blood draws where the male driver was charged with the offense of driving while intoxicated.

This presentation will impact the forensic community and/or humanity by providing additional data that can be used by testifying forensic scientists in determining more accurate estimations of blood alcohol levels in drivers at the time of the incident.

Ethanol intoxication is a leading cause of motor vehicle accidents in the U.S. with a reported rate of alcohol involvement in 39% of all traffic fatalities in 2005[1]. During the same time frame in Texas, 46% of all traffic fatalities involved alcohol.[1] The Federal Bureau of Investigation estimated that about 1.4 million drivers in the U.S. were arrested in 2005 for driving under the influence of either narcotics or alcohol with males accounting for 81% of these arrests.[2, 3]

Current Texas law states that any accident resulting in serious bodily injury or loss of life allows for the collection of a suspect's blood for toxicological analysis. Since 2002, the Bexar County District Attorney's Office has requested that two blood specimens be obtained with an intended elapsed time interval of two hours between the blood draws. For the cases studied, from the years 2003-2007, the actual average elapsed time was 104 minutes. Blood draws were taken at local hospitals and transported to the Bexar County Medical Examiner's Office under the chain-of-custody by the arresting officer. The blood samples were then analyzed for ethanol concentrations using a direct-injection gas chromatography (GC) method.

Sample Preparation: 0.2 mL of sample blood was added to 4.0 mL of the internal standard (IS) solution, using a Repipet dilutor (LabIndustries, Dubuque, IA). The IS solution was composed of 0.25 mL n-propanol brought up in 1 L of deionized water (0.025% v/v). The analyte solution spiked with the IS was then transferred to a GC vial and loaded onto the injection tray.

Analysis: The samples were then analyzed on a Hewlett Packard 6890 GC. The method utilized an isothermal oven temperature of 40°C and a run time of 3 minutes. The gas chromatographic column employed was the Restek Rtx – BAC1 (30m x .53mm id, 3µm film thickness). The carrier gas was helium at a velocity of 11.2 mL/min and detection was accomplished by a flame ionization detector. Autoinjection and collection parameters were controlled by Agilent GC ChemStation software.

Ethanol elimination rates were calculated using the following:

$$\frac{[BAC]_1 - [BAC]_2}{\Delta T}$$

where *[BAC]* represents the reported ethanol concentrations in g/dL and ΔT equals the elapsed time between the two draws in hours.

Results: Ethanol was not detected in six cases out of the 173 studied and thus were excluded from data analysis. The range of calculated ethanol elimination rates were 0.0005 to 0.0682 g/dL/hr. The mean, median, and mode ethanol elimination rates were 0.0198, 0.0175, and 0.0175 g/dL/hr, respectively. Initial blood alcohol concentrations reported in the study ranged from 0.018 to 0.397 g/dL. Table 1 describes the relationship between the age of the male subject and the elimination rate while Table 2 examines how the elimination rate varies with a change in the initial blood alcohol concentration.

Table 1.

Age Range	N	Average Elimination Rate (g/dL/hr)
16-19	20	0.0194
20-29	73	0.0204
30-39	29	0.0156
40-49	27	0.0198
50+	18	0.0246
All Cases	*167*	*0.0198*

Table 2.

Initial BAC Range (g/dL)	N	Average Elimination Rate (g/dL/hr)
≤ 0.0499	6	0.0156
0.05-0.099	18	0.0170
0.1-0.149	42	0.0184
0.15-0.199	50	0.0201
0.2-0.249	35	0.0205
≥ 0.25	16	0.0248

No correlation was observed between a person's age and their elimination rate, however an increase in the rate of ethanol elimination was observed with increasing initial blood alcohol concentrations.

The overall variability of the elimination rates can be attributed to a mixture of genetic and acquired factors such as decreased enzyme activity, gastric contents, as well as a difference between the time of the incident and the first blood draw.

References:
1 http://www-nrd.nhtsa.dot.gov/pdf/nrd-30/NCSA/TSF2005/810616.pdf
2 http://www.fbi.gov/ucr/05cius/data/table_29.html
3 http://www.fbi.gov/ucr/05cius/data/table_41.html

Ethanol, Elimination Rate, Impaired Driving

K24 A Fast and Sensitive LC/MS/MS Method for the Quantitation and Confirmation of 30 Benzodiazepines and Non-Benzodiazepine Hypnotics in Forensic Urine Samples

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After attending this presentation, attendees will learn about using

LC/MS/MS for analysis of benzodiazepines and non-benzodiazepine hypnotics in urine. These drugs are of interest because of increased use and abuse.

This presentation will impact the forensic science community by presenting a fast, simple, and sensitive technique to detect and quantify benzodiazepines and other hypnotic drugs in urine. The method presented has several advantages over the other techniques that are used.

Introduction: Benzodiazepines and other nonbenzodiazepine hypnotics, such as Zaleplon, Zolpidem, and Zopiclone are widely prescribed psychoactive drugs for the treatment of anxiety and sleep disorders. These substances frequently lead to dependence and abuse and some of them can affect judgment and behavior. As a result, these compounds are of great interest in forensic, toxicological and clinical research laboratories. The screening for benzodiazepines with immunoassay tests does not provide enough sensitivity and specificity. Analysis using gas chromatography with different detectors is difficult or impossible because of thermal instability and requires time consuming derivatization and clean-up steps. Liquid chromatography (LC) with UV detection cannot detect benzodiazepines at required concentration levels and lacks in selectivity.

LC with tandem mass spectrometric detection (MS/MS) with electrospray ionization (ESI) is the ideal technology for the analysis of polar and thermally labile drugs and their metabolites, yielding high sensitivity and specificity. Sample preparation is also fast and simple. The developed LC/MS/MS method detects 30 analytes in a single chromatographic run using two Multiple Reaction Monitoring (MRM) transitions to allow quantitation and confirmation.

Experimental and Results: Urine samples of forensic cases were diluted after addition of internal standards. LC separation was carried out using a Shimadzu Prominence LC using mobile phases of: (A) water with 0.2% formic acid and 2mM ammonium formate, and (B) acetonitrile with 0.2% formic acid and 2mM ammonium formate. A 3200 Q TRAP® LC/MS/MS system equipped with an ESI source operated in the MRM mode was used for detection. Two transitions were monitored. The first MRM was used to quantify the analyte and a ratio of the quantifier to the second qualifier MRM was used for confirmation.

Limits of quantitation in urine samples, accuracy and reproducibility of all analytes were studied. All targeted compounds could be quantified in urine samples after a dilution step at a concentration of at least 10 ng/mL. The high selectivity of MRM detection allows compound specific detection without interference of urine matrix or other drugs or metabolites being present in the sample. The dilution step of the urine sample preparation additionally ensures elimination or reduction of ion suppression which could be caused because of co-eluting matrix components.

Benzodiazepines, LC/MS/MS, Toxicology

K25 Disposition of MDMA and Metabolites in Human Sweat Following Controlled MDMA Administration

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After attending this presentation, attendees will learn that understanding the disposition and excretion of methylenedioxymethamphetamine (MDMA) and metabolites in the sweat of MDMA (ecstasy) users is vital for interpreting sweat and hair testing results in drug treatment, criminal justice and workplace drug testing programs.

This presentation will impact the forensic science community by demonstrating how this experimental MDMA administration study indicates that sweat testing may be an effective and reliable method for monitoring ecstasy use. These data provide a scientific database for interpretation of MDMA sweat test results.

Placebo, low (1.0 mg/kg) and high (1.6 mg/kg) oral MDMA were given double blind in random order to healthy volunteers (n=16) with a history of MDMA use. Participants provided written informed consent to participate in this IRB-approved study and remained on a closed clinical research unit for at least three days after each MDMA dose. PharmChek® sweat patches (n=688) were worn prior to dosing, reflecting previously self-administered drug, and during and after controlled MDMA dosing. Patches were analyzed by SPE and GC/MS for MDMA, methylenedioxyamphetamine (MDA), 4-hydroxy-3-methoxyamphetamine (HMA) and 4-hydroxy-3-methoxymethamphetamine (HMMA). Limits of quantification (LOQ) were 5 ng/patch except for MDMA (2.5 ng/patch).

MDMA was the primary analyte detected with concentrations up to 3007 ng/patch in 415 patches (60.3%). MDA was detected in 194 patches (28.2%) at concentrations <172 ng/patch, and HMA, and HMMA were not detected above the method LOQ. 234 patches (34.0%) were positive for MDMA at the 25 ng/patch screening and confirmation cutoffs, proposed by the Substance Abuse and Mental Health Services Administration (SAMHSA) for the detection of amphetamines. Four additional patches (0.6%) exceeded these cutoff concentrations for MDA, and only one was positive without concurrent MDMA above the method LOQ.

MDMA was first observed in short-term patches worn from 0-2.5 h after low and high dose administrations. MDA was present in patches worn 0-6 h after dosing. Large intra- and inter-subject variability was observed in duplicate weekly patches applied for seven days. Median weekly MDMA concentrations were 137.5 ng/patch (5.8 - 894.0) and 376.6 ng/patch (14.7–3007.7) following low (n=18) and high (n=23) doses, respectively.

This research is supported by the Intramural Research Program, NIH, National Institute on Drug Abuse.

MDMA, Sweat, GC/MS

K26 A Fatal Case of a Paint Thinner Ingestion: Comparison Between Toxicological and Histological Findings

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The goal of this presentation is to illustrate a fatal case of self-poisoning by ingesting of solvents used to dilute varnishes.

This presentation will impact the forensic science community by evaluating the histopathological findings in liver, kidney, and brain slides with the results reported in scientific literature about pathologic pathways found in animal models exposed to acute solvents.

The authors illustrate a fatal case of self-poisoning by ingesting of solvents used to dilute varnishes.

A 15-year-old Caucasian boy was found in supine position in the garden of his teacher's country-house. Past history indicated that the boy went to confront his teacher about a recent failed examination in his class. He was transported to the local Emergency Room and died shortly afterwards. During the subsequent death investigation, police officers found numerous small bottles of paint thinners. By an order of the legal authorities, an external examination and autopsy were performed two days later at the Institute of Legal Medicine of Palermo.

External examination: The young boy was 175 cm tall and weighed 65 kg. No injuries were found on his body; the external examine showed only a nasal haemorrhage and labial and subungual cyanosis.

Autopsy findings: The forensic autopsy revealed citotoxic wet brain

and congestion of cerebral veins. There were no lesions on the scalp or in the galea capitis and no intracerebral haemorrhaging was found. Pulmonary edema, pancreas and kidney congestion were found. The gastric content consisted of a brownish liquid (300 cc) and its odour suggested the presence of organic volatiles. Nothing else was found during the autopsy.

Histological findings: The microscopic examination showed a multi-visceral congestion. The oesophageal mucous membrane showed multiple lympho-granulocyte infiltrates.

Toxicological analysis: The blood alcohol screening was negative. Additional toxicological analysis revealed the presence of toluene and *ortho*-, *meta*-, *para*- xylene. Analysis was performed by the Headspace/Solid-Phase Microextraction/Gas Chromatography-Mass Spectrometry (HS-SPME-GC/MS) to identify and quantify volatile organic compounds in blood and tissue samples. Experimental condition included headspace sampling at 40°C. Carboxen/PDMS fiber (85 mm) repeatedly tested at various adsorbing and desorbing times in order to obtain the best compromise in term of chromatogram quality and method sensitivity. The capillary column used was SUPELCOWAXTM 10. The quantitative analysis was carried out using toluene-d8 as the internal standard. In order to optimize the GC-MS method, a preliminary study was conducted using a single quadrapole instrument in SIM mode. The first quantitative data on blood were: toluene 60 mg/L, *ortho*- xylene 232 mg/L, *meta*- xylene 160 mg/L, *para*-xylene 65.2 mg/L. Afterwards, the analysis on the other tissue samples (gastric contents, brain, etc.) were performed using a GC-MS equipped with an ion trap mass analyzer and an autosampler, in order to achieve a better reproducibility on data and reduce manual errors. Ions at m/z 91, 106 and m/z 98 (i. std. toluene-d8) were used to quantify the aromatic compounds both in SIM mode using a single quadrapole instrument and, as extracted ions, in SCAN mode using a ITD mass analyzer.

Solvents Ingestion, Self-Poisoning, Organic Volatile Toxic

K27 A Review of Cases Analyzed for 1,1-difluoroethane

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After attending this presentation, attendees will learn the bodily fluid and tissue concentrations of 1,1-difluoroethane and understand the relationship of demographic information to 1,1-difluoroethane abusers.

This presentation will impact the forensic toxicological and pathological communities by presenting data on 1,1-difluoroethane concentrations in human fluids and tissues where there is currently a relative dearth of current information.

1,1-difluoroethane (DFE) is a colorless, odorless gas used as a refrigerant and as an aerosol propellant in many commonly used consumer products and electronic cleaners. Over the past few years it has been recognized as a substance of abuse that can lead to injury or death. In general, inhalation of fluorinated hydrocarbons may result in a feeling of light-headedness and disorientation; however, in higher concentrations abuse may lead to cardiac dysrhythmias and sudden death.

A review of cases from our laboratory database for the past two years revealed 48 cases for which DFE was analyzed. The analysis for these specimens was performed by headspace gas chromatography/mass spectrometry. All but three of the cases found DFE to be present. The specimens in which DFE was detected included blood (n=35) (while most did not identify the source of the blood some were identified as central, chest, iliac, inferior vena cava, femoral or peripheral); lung tissue (n=8), brain tissue (n=2); adipose tissue (n=1); and urine (n=1). The blood concentrations ranged from 0.14 to 300 mcg/mL, average (± SD) 51 ± 78 mcg/mL and median 23 mcg/mL. In most cases, our laboratory did not receive case histories with the accompanying specimens; therefore, it was difficult to determine if the blood tested was an investigation of a death (due to an overdose or an accident), investigation of human performance involving an accident, or probable cause for substance abuse. However, based on the source of the specimen and/or the submitting client, 21 blood specimens were identified as postmortem blood that had DFE concentrations ranging from 0.74 to 300 mcg/mL, average (± SD) 79 ± 91 mcg/mL, and median 45 mcg/mL. The DFE concentration in the lung tissues examined ranged from 0.86 to 59 mcg/g, average (± SD) 20 ± 24 mcg/g, and median 11.3 mcg/g. The two brain tissues had concentrations of 26 and 100 mcg/g. The one adipose tissue and one urine sample had DFE concentrations of 6.8 mcg/g and 0.94 mcg/mL respectively. In three cases where multiple samples were analyzed, the DFE concentrations were blood 65 mcg/mL and brain tissue 100 mcg/g; blood 23 mcg/mL and lung tissue 0.94 mcg/g; and adipose tissue 68 mcg/g and lung tissue 24 mcg/g. It should be noted that DFE is a gaseous substance and may volatilize on handling; therefore, the reported values may be lower than the circulating concentrations.

In 37 of the cases where the gender was identified, 73% (27) were male and 27% (11) were female. The ages of the individuals were given in 35 of the cases and ranged from 15 to 55 years, average (± SD) 27 ± 10 years, and median 25 years. In cases were both gender and age were noted, the average (± SD) age of the males (n=24) was 29 ± 11 years (range 15 – 55 years), median 27.5 years; and the average (± SD) age of the females (n=9) was 22 ± 6.6 years, (range 16 – 31 years), median 18 years. In conclusion, DFE can be found in a variety of bodily fluids and tissues in a forensic toxicological investigation and appears to be predominantly abused by men in their late twenties, but also can be found in women usually of a younger age. With the prevalence of this propellant in commonly used consumer products, the abuse of this substance will likely continue.

1,1-difluorethane, Toxicology, Inhalant Abuse

K28 Evaluation of the Lin-Zhi International Benzodiazepine Enzyme Immunoassay for the Detection of Benzodiazepines and Their Metabolites in Urine

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The goal of this presentation is to inform the toxicology community and others of the performance of the Lin-Zhi International Benzodiazepine Enzyme Immunoassay for the detection of benzodiazepines and their associated metabolites in urine.

An evaluation of the performance of the Lin-Zhi International Benzodiazepine Enzyme Immunoassay will impact the forensic science community by providing the field of toxicology with an alternative choice for the rapid detection of benzodiazepines and their associated metabolites in urine.

An evaluation of a new Benzodiazepine Enzyme Immunoassay [BEI] (Lin-Zhi International, Inc., Sunnyvale, CA) for the detection of benzodiazepines and their major metabolites in urine will be presented. The Lin-Zhi assay is based on competitive antibody binding between benzodiazepines in urine and glucose-6-phospahate dehydrogenase labeled oxazepam. When benzodiazepines and/or their metabolites are present in urine, active unbound enzyme reduces the co-enzyme NAD to NADH that results in an increase of measured absorbance at 340 nm. The assay is calibrated with oxazepam.

The BEI was evaluated by testing 1409 urine specimens collected from pain management patients. All 1097 specimens were tested with the assay in an ADVIA 1200 Chemistry System auto-analyzer (Bayer Health Care, Diagnostics Division, Tarrytown, NY) with calibrators containing 0, 200 (cut-off calibrator) and 600 ng/mL of benzodiazepine. Controls containing 0 ng/mL of oxazepam and -25% (negative control) and +25% (positive control) of the 200 ng/mL cut-off calibrator (Bio-Rad Laboratories, Irvine, CA)

were analyzed with each batch of samples. All urines were then analyzed by a GC/MS for alprazolam, hydroxy-alprazolam, diazepam, nordiazepam, lorazepam, oxazepam and temazepam at a cut-off concentration of 75 ng/mL.

Approximately, 30% (315) of the 1097 specimens yielded positive results by the BEI assay. Of these specimens, GC/MS confirmed the presence of Benzodiazepines at 75 ng/mL in 306 specimens, indicating 9 false positive results. However, 36 specimens yielding negative BEI results were found to contain Benzodiazepines above the GC/MS cut-off of 75 ng/mL. Therefore the overall agreement of BEI and GC/MS results was 96%. From the presented study, the sensitivity of the BEI was 0.895 and the selectivity 0.988. Testing at 1,000 mg/mL of other drugs of abuse or their metabolites such amphetamine, benzoylecgonine, morphine and phencyclidine, BEI demonstrated no cross reactivity. The within-run precision of BEI was determined by the absorbance rates of the negative and positive controls was CV=1% (n=16); while the between-run precision of the controls was CV=<6% (n=16). The assay was found linear from –50% to 150% of cut-off concentration. The Lin-Zhi BEI provides a precise, reliable method for the routine detection of benzodiazepines and/or their metabolites in urine specimens.

Enzyme Immunoassay, Benzodiazepines, Urine Drug Testing

K29 Evaluation of the Lin-Zhi International Phencyclidine Enzyme Immunoassay for the Detection of Phencyclidine in Urine

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The goal of this presentation is to inform the toxicology community and others of the performance of the Lin-Zhi International Phencyclidine Enzyme Immunoassay for the detection of Phencyclidine in urine.

An evaluation of the performance of the Lin-Zhi International Phencyclidine Enzyme Immunoassay will impact the forensic science community by providing the field of toxicology with alternative choices for the rapid detection of phencyclidine in urine.

An evaluation of a new Phencyclidine Enzyme Immunoassay [PCPI] (Lin-Zhi International, Inc., Sunnyvale, CA) for the detection of phencyclidine (PCP) in urine will be presented. The Lin-Zhi assay is based on competitive antibody binding between PCP in urine and glucose-6-phospahate dehydrogenase labeled PCP. When PCP is present in urine, active unbound enzyme reduces the co-enzyme NAD to NADH that results in an increase of measured absorbance at 340 nm.

The PCPI was evaluated by testing 412 urine specimens collected from criminal justice clients and substance abuse treatment patients. All 412 specimens were tested with the assay in an ADVIA 1200 Chemistry System auto-analyzer (Bayer Health Care, Diagnostics Division, Tarrytown, NY) with calibrators containing 0 and 25ng (cut-off calibrator) of PCP. Controls containing 0 ng/mL of PCP and -25% (negative control) and +25% (positive control) of the 25 ng/mL cut-off calibrator (Bio-Rad Laboratories, Irvine, CA) were analyzed with each batch of samples. All urines were then analyzed by HPLC-MS/MS for PCP at a cut-off concentration of 5 ng/mL.

Approximately, 29% (118) of the 412 specimens yielded positive results by the PCP assay. Of these specimens, HPLC-MS/MS confirmed the presence of at 5 ng/mL in 118 specimens, indicating no false positive results. Only one specimen yielded a negative result and was found to contain PCP above 25 ng/mL. Therefore, the overall agreement of PCPI and HPLC-MS/MS results was 99.8%. From the presented study, the sensitivity of the PCPI was 0.992 and the selectivity 1.000. Testing at 100 mg/mL of other drugs or their metabolites such as amitriptyline, amphetamine, benzoylecgonine, diphenhydramine, doxepin, doxylamine, imipramine, morphine, and oxycodone PCPI demonstrated no cross reactivity. The within-run precision of PCPI was determined by the absorbance rates of the negative and positive controls was CV=2% (n=12); while the between-run precision of the controls was CV=<6% (n=4). The assay was found linear from –50% to 150% of cut-off concentration. The Lin-Zhi PCPI provides a precise, reliable method for the routine detection of phencyclidine in urine specimens.

Enzyme Immunoassay, Phencyclidine, HPLC/MS/MS

K30 Identification of Product Adulteration With Pesticides Via Direct Analysis in Real Time (DART™) Time-of-Flight Mass Spectrometry (TOF-MS)

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The goal of this presentation is to demonstrate that DART-TOF-MS instrumentation is a beneficial counterpart in the modern forensic laboratory to traditional toxicological examinations.

This presentation will impact the forensic science community by demonstrating how Direct Analysis in Real Time (DART) ionization sources may provide a more rapid and direct analytical route to toxicant identification in adulterated food or beverage specimens.

DART™ stands for "Direct Analysis in Real Time". A new ion source, DART™ allows for the direct detection of chemicals on surfaces, in liquids, and in gases without the need for sample preparation. The ion source is open to the atmosphere, and does not require a vacuum, the use of high voltages or solvent sprays. Samples are simply placed into the ionization stream. The ionization mechanism is based upon the reactions of excited-state species with reagent molecules and polar or non-polar analytes. When coupled with a TOF-MS, accurate mass assignments are generated and analyte identifications are realized.

In many toxicological investigations, the suspected source of the poison may be received in addition to biological specimens such as blood or urine. Often, the most direct route to toxicant identification is primary analysis of suspect source material. However, analysis of seized food, beverage, and other commercial products is typically time consuming and laborious. A DART-TOF-MS instrument offers the forensic examiner a tool for rapidly identifying such adulterations in bulk samples. Such analyses can complement subsequent traditional investigations by immunoassay and chromatography.

Several cases are presented in which beverages or food products were suspected to have been spiked with a commonly available pesticide formulation. Commercially available herbicide formulations often contain a variety of organochlorine, organophosphorus or other compounds. Ingestion of such preparations may cause severe injury or death.

Direct analysis of both the seized food samples and a pesticide exemplar by DART-TOF-MS yielded a rapid and conclusive identification. The analysis provided exact molecular mass, theoretical isotope distribution matching, and characteristic fragmentation patterns to effect the identifications. No sample preparation was required. The pesticides identified were

2,4-D, dicamba, mecoprop, and diethyltoluamide.

DART-TOF-MS, Pesticides, Accurate Mass

K31 National, Regional, and State Trends in Workplace Urine Drug Testing Results From a Medical Review Officer Data Source, 2003-2005

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After attending this presentation, attendees will have an enhanced understanding of the relationships between laboratory reported drug test results and Medical Review Officer (MRO) verified results reported to employers from 2003-2005 for Non-Federally Regulated Workplace Drug Testing. Drug testing results will be evaluated nationally, by census region, and by state. Map displays by state will evidence variation across states. Geographic Information System (GIS) functionality will display spatial distribution of the data.

This presentation will impact the forensic science community by describing how annual positive rates by geographic area for the Non-Regulated Workforce determined from Medical Review Officer (MRO) verified data, which excludes blind quality assurance samples and results reversed through valid medical explanation, may more accurately represent illegal drug use rates and geographical trends to the forensic community.

Records for over 2.1 million Non-Regulated specimens collected during calendar years 2003 through 2005 from more than 8,000 companies and tested by 41 laboratories were obtained from a large MRO data source. The database includes donor demographics, employer information, collection site information, laboratory results, and MRO determinations, but does not include employer blind quality assurance samples. Analysis of the data indicates that, following MRO review, there was a decreasing trend of annual positive rates and an increasing trend of reversal rates in regulated and non-

Non Regulated Drug Testing 2003-2005	Total, U.S.	Northeast	South	Midwest	West
MRO Verified Drug Positive Rates, %					
Overall Drug Positive Rate, 2003	3.74%	3.39%	3.56%	4.40%	3.71%
Overall Drug Positive Rate, 2004	3.52%	3.31%	3.52%	3.58%	3.63%
Overall Drug Positive Rate, 2005	3.18%	3.07%	3.21%	3.21%	3.16%
MRO Verified Drug Positive Rates, %					
Overall Drug Positive Rate, 2003-2005	3.46%	3.25%	3.41%	3.70%	3.46%
Amphetamines	0.18%	0.05%	0.14%	0.11%	0.51%
Cocaine	0.61%	0.59%	0.68%	0.63%	0.37%
Marijuana	2.49%	2.44%	2.39%	2.78%	2.44%
Opiates	0.08%	0.07%	0.09%	0.09%	0.08%
Phencyclidine	0.01%	0.03%	0.01%	0.01%	0.01%
Barbiturates	0.06%	0.07%	0.08%	0.04%	0.04%
Benzodiazepines	0.14%	0.09%	0.19%	0.11%	0.11%
# of Specimens Tested, 2003-2005	2,111,528	320,329	999,052	443,807	348,340

regulated populations during the 36 month period. The table below illustrates the geospatial distribution of MRO verified positive drug testing results for Non-Regulated specimens by U.S. Census regions.

The overall MRO verified drug positive rate has decreased nationally as well as in all four regions during the period 2003-2005. The most dramatic decrease was in the Midwest region where it decreased from 4.40% to 3.21%. Looking at drug classes for 2003-2005, the MRO verified positive rate for amphetamines was significantly greater in the West region (0.51%), while the cocaine positive rate in the West (0.37%) was the lowest of all regions. The positive rates for marijuana and opiates were not significantly different among the regions. The positive rates for both barbiturates and benzodiazepines were highest in the South region. The annual positive rate for marijuana has consistently evidenced a downward trend nationally (2.76% to 2.21%) and in all regions during the three year period. Data supports the observation that Workplace Drug Testing may be a deterrent to illicit drug use.

Workplace Drug Testing, Drug Testing Database, Medical Review Officer Verified Results

K32 A Stability Study on Ritalinic Acid in Urine

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The goal of this presentation is to evaluate the short-term stability of ritalinic acid in authentic urine specimens exposed to different storage conditions.

This presentation will impact the forensic science community by identifying adequate storage conditions for urine samples obtained from methylphenidate users; and contribute to proper interpretation of results from delayed analyses or reanalyses by reviewing indications from the assessment of ritalinic acid stability.

The stability of any drug and potential metabolites in biological samples must be considered when justifying the reliability of analytical results. Variation in drug concentrations in biological fluids is possible via thermal, chemical, enzymatic or matrix degradation. Stability studies can improve toxicological quality by identifying optimal storage conditions and time limits for analysis, after sample collection, and reanalysis. The examination of the short-term stability of ritalinic acid, assessed through quantitative results for eight positive ritalinic acid urine samples obtained from pain management patients prescribed methylphenidate, is presented.

Ritalinic acid is the primary metabolite of methylphenidate, a phenethylamine derivative employed in the treatment of attention-deficit hyperactivity disorder (ADHD), childhood hyperkinesis, depression and narcolepsy. Previous research has revealed that methylphenidate spontaneously hydrolyses to ritalinic acid in vitro.[1] The conclusion from that scientific study recommends freezing or refrigeration conditions for specimen storage. Some commercial laboratories refuse analysis of specimens that have not been frozen. The methylphenidate degradation process is minimized at pH 2.9, or by addition of ethylenediaminetetraacetic acid (EDTA), and under "cool" storage.[1] Studies have confirmed the percentage of methylphenidate in urine to be minimal, less than 1% in a twenty four hour void.[2, 3] Consequently, urine methylphenidate concentrations are usually quite low and if conversion to ritalinic acid does occur, it is unlikely that the ritalinic acid content will be significantly increased. There have been no published studies on ritalinic acid stability to date.

The ritalinic acid concentration in three sets of urine aliquots from authentic cases (n=8), stored at room temperature, refrigerated or frozen was quantified by gas chromatography/mass spectrometry (GC/MS), once weekly, over a one-month period. The baseline concentration range of all specimens was 10,945-78,673 ng/mL. Loss of analyte appears to be concentration dependent. Deterioration is more rapid at higher ritalinic acid concentrations. There was a significant decrease in concentration of the target analyte over the twenty nine-day period; mean percentage loss of analyte was 32%, 36%, and 43% for highly concentrated ritalinic acid specimens (46,332-78,673 ng/mL) stored at room temperature, refrigerated and frozen, respectively. No statistically significant difference in the variation of ritalinic acid content among the samples stored under the three conditions was evident. At lower ritalinic acid content (10,945-18,594 ng/mL), change in concentration was insignificant. All statistical analysis was done at the 95 % confidence limit; P= 0.05. The results indicate that ritalinic acid is unstable in urine, particularly at high concentrations, and the

concentration will decrease significantly upon storage at room temperature, refrigeration, or freezing.

Stability, Ritalinic Acid, Storage Condition

K33 Methadone Detection in Postmortem Oral Swab Samples

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After attending this presentation, the attendees will have an understanding of the use of oral swab samples obtained after death for the detection of methadone.

The presentation will impact the forensic community and/or humanity by demonstrating how oral swabs may be valuable in establishing methadone use in cases of fatal drug overdose.

Recent studies have shown that oral fluid samples are useful for detecting drug use. The purpose of this study was to evaluate whether a specific drug, methadone, could be detected in oral swab samples obtained after death. At present, methadone is among the most commonly detected drugs in fatal drug overdoses in West Virginia.

Oral swabs were obtained in cases in which the cause of death was suspected to be drug-related. Autopsy technicians collected the samples using standard laboratory cotton-tipped swabs by rubbing the swab along the buccal mucosa. Samples were eluted by vortexing with 1.0 mL of methanol and were centrifuged to remove debris. The supernatant was collected, dried under nitrogen, and reconstituted in 0.10 mL of methanol. GC/MS analyses were performed using an Agilent 6890 gas chromatograph interfaced with a 5973 mass-selective detector.

Saliva collected from non-methadone using donors was used for validation with deuterated methadone as an internal standard. Preliminary experiments demonstrated that an unidentified substance present in the swabs co-eluted with methadone. Several attempts were made to modify GC parameters in order to circumvent this interference. These were unsuccessful in resolving the two compounds; therefore, selected-ion monitoring was employed for analysis of methadone in the swabs. Target and qualifier ions acquired for methadone and deuterated internal standard were: methadone 72, 294, 223; methadone-*d9* 78, 226,178. The analysis had a linear range of 36.5 ng/swab to 365 ng/swab (r^2=0.991) and a limit of detection of 29.2 ng/swab. Precision of the assay was demonstrated with intraday (n=3) and interday (n=3) coefficients of variation of 5.72% and 13.4%, respectively, using a control containing 292 ng/swab. Average methadone recovery was 27.3% when spiked saliva (0.1 mL) was added to the swab.

Cases that were confirmed to be methadone-positive and quantitated in blood were chosen for the study. The average weight of material collected on the swab was 83 mg ± 41 mg.

All calibrators and controls were required to be ± 20% of their intended values. Twenty-six case samples were analyzed with a maximum of five case samples included in each assay. Assays also included four calibrators (36.5, 73.0, 183, 365 ng/swab), two positive controls (54.8, 292 ng/swab), and one negative control (saliva with no methadone added).

Methadone was detected in 17 of the 26 samples, three of which were below the LOQ (< 36.5 ng/swab). The amount of methadone in the samples varied from 38.9 to 333 ng/swab. The methadone metabolite, EDDP, was not studied, but it was noted that methadone was not detected in any of the swab samples from cases for which EDDP in blood was found to be below our limit of detection, 0.01 mg/L.

Methadone, Oral Fluid, Postmortem

K34 Analyzing Cannabiniods by HPLC/MS/MS

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After attending this presentation, attendees will understand how to increase sample throughput using HPLC/MS/MS methodology and how to chose the proper method conditions to obtain reliable, enhanced sensitivity for cannabinoid analysis.

The HPLC methodology discussed will impact the forensic science community by providing an alternate means of analyzing cannabinoids at low concentrations compared to the current GC methodology.

After attending this presentation, attendees will understand how to increase sample throughput using HPLC/MS/MS methodology and how to chose the proper method conditions to obtain reliable, enhanced sensitivity for cannabinoid analysis. The HPLC methodology discussed provides the forensic community with an alternate means of analyzing cannabinoids at low concentrations compared to the current GC methodology.

This study included developing an HPLC/MS/MS method for analyzing cannabinoids. The main psychoactive component in marijuana, Δ^9 – tetrahydrocannabinol (Δ^9–THC), is quickly absorbed and metabolized to 11-hydroxy-Δ^9-tetrahydrocannabinol (hydroxy-THC), an active metabolite. The hydroxy-THC is further metabolized (rapidly) to 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (carboxy-THC), an inactive metabolite commonly found in urine, blood, hair, and other tissues. GC-MS (Gas Chromatography-Mass Spectrometry) often is used for confirming and quantifying Δ^9–THC and carboxy-THC. However, GC-MS methods require time-consuming steps like derivatization to obtain acceptable chromatography. Using HPLC (High Performance Liqui Chromatography), derivatization is eliminated, saving time without sacrificing sensitivity.

A quantitative method for analyzing underivatized cannabinoids by HPLC tandem mass spectrometry was developed. Goals were threefold in this study; 1) to optimize the column selection, 2) to provide a short analysis time and, 3) to obtain reliable confirmation and quantitation data in the low ng range (< 10ng).

Results showed that choosing a column that produced longer retention allowed for the use of a high organic mobile phase composition. This high organic mobile phase composition increased desolvation efficiency and enhanced sensitivity of the cannabinoids. Detection at the picogram level was obtained. The high organic mobile phase composition also contributed to a short analysis time of 5 minutes. The use of the MS/MS instrumentation produced reliable identification by producing two +MRM transitions.

Based on the work described above, a biphenyl HPLC column coupled to an HPLC MS/MS can quantify low levels of analyte from underivatized sample – and can achieve baseline separation of Δ^9-THC and cannabidiol (which have very similar product ion spectra and +MRM transitions) – in less than 5 minutes.

Mass Spectrometry, HPLC, THC

K35 The Advantages and Limitations of MRM vs. Full Scan MS/MS for Drug Confirmation Utilizing LC/MS/MS

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After attending this presentation, attendees will become familiar with two methods of drug screening using liquid chromatography tandem mass spectrometry (LC/MS/MS) and will understand the pros and cons of using full scan MS/MS versus two multiple reaction monitoring (MRM) transitions for drug confirmation.

This presentation will impact the forensic community by adding data to the debate of what constitutes a confirmation of drug presence in LC/MS/MS.

Introduction and Hypothesis: GC/MS has been the analytical technique of choice for drug confirmation in forensic toxicology labs. However, the use of LC/MS/MS for screening and confirmation has been increasing and this technique continues to be adopted by a rising number of labs. When any new confirmatory technique is implemented, debates arise regarding what constitutes a confirmation. Although it has been established that three ions are necessary for GC/MS SIM confirmation, the criteria for an LC/MS/MS confirmation is still a highly debated topic. In this work, confirmation using two MRM transitions is compared and contrasted with confirmation using full scan MS/MS spectra. The advantages and limitations of both techniques are presented and discussed. The goal of the study was to investigate which LC/MS/MS method was more robust and which had the largest dynamic range for drug confirmation.

Methods: Standards of various drug compounds were spiked into drug free urine and diluted 10x with mobile phase. Analysis was performed on an LC interfaced to a hybrid triple quadrupole/linear ion trap (LIT) mass spectrometer (Applied Biosystems 3200 QTrap). All compounds were analyzed using positive mode electrospray ionization.

For the MRM only method, two MRM transitions per analyte were monitored with the second transition functioning as a qualifier ion. The ratio of the peak areas of the target MRM to the qualifier MRM was calculated. For confirmation, it was required that the ratio be within +/- 20% of the standard.

When full scan MS/MS spectra were used for confirmation, an MRM survey scan was used to detect the presence of an analyte. If an analyte was detected, the system automatically acquired a full scan MS/MS spectrum of the compound using Q3 operating in LIT mode. The resulting spectrum could be searched against a library for identification and confirmation. A purity match of about 70% or higher was required for confirmation.

The precision for the two methods was also compared since it was expected that the full scan method may compromise precision due to switching between MRM and full scan modes.

Preliminary Data: Preliminary results from analysis of amphetamines showed that a full scan spectrum using LIT was more robust in confirmation than using a ratio of two MRM transitions. In 15 samples at 100 ng/mL, amphetamine and methamphetamine passed every time in both methods, MDA failed in both methods, and MDMA passed in the full scan method but failed 13 out of 15 times in the MRM with qualifier method. Also, there was no significant compromise in precision with the full scan MS/MS method: the within run and between day precision was 4.6 and 6.0, respectively, compared to 3.8 and 5.8 for the MRM with two transitions method.

Conclusion: To conclude, initial findings indicate that both the full scan method using a LIT and the MRM with two transitions method are robust and precise in performing amphetamine confirmations. However, the full scan method was more robust at the low end of the dynamic range. This study will be expanded to include drugs from other classes to determine if the initial trends are observed across most compounds and to determine how well each method functions in situations of co-eluting drugs at large concentration ranges.

LC/MS/MS, Drug Screening, Drug Confirmation

K36 Correlation Study Between Blood Concentrations and Vitreous Concentrations: Case of Meprobamate and Some Benzodiazepines (Bromazepam, Nordazepam, Oxazepam)

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The goal of this presentation is to show that, for the studied population, there is a positive correlation between blood and vitreous concentrations of meprobamate. There is no positive correlation for bromazepam, nordazepam and oxazepam. For all the molecules studied, concentrations measured in the vitreous, collected in the right and left eye, are not different.

This presentation will impact the forensic science community by confirming that vitreous concentrations of benzodiazepines cannot be used to extrapolate blood concentrations. For meprobamate, the results of this study show that the vitreous concentrations may be used to calculate blood concentration. The forensic toxicologist can provide a reliable result of quantitative analysis even if only one of the two vitreous is available.

Blood sample is the gold standard for most of the drugs in forensic toxicology. However the blood sample could be damaged or inexistent. In those cases, vitreous could be an alternative sample. It is thus important to know the relationship between blood and vitreous concentrations. This study has been performed on molecules often involved in forensic toxicology: meprobamate and some benzodiazepines (bromazepam, nordazepam, oxazepam).

Blood and vitreous samples were collected during forensic autopsies. Included in this study were the cases where one or many of the studied molecules (bromazepam (n=31), nordazepam (n=58), oxazepam (n=28) and meprobamate (n=43)) were detected in blood or urine during routine toxicology investigations. Benzodiazepines were quantified in blood by HPLC/DAD and in vitreous by Ultra Performance Liquid Chromatography with UV detection (UPLC/DAD). Meprobamate was quantified in blood and vitreous by GC/MS.

No difference has been highlighted, for all the molecules studied, between the vitreous collected in the right and left eye. A significant correlation (r^2=0.86) has been highlighted for the meprobamate. No correlation has been found for bromazepam (r^2=0.32), nordazepam (r^2=0.45), oxazepam (r^2=0.52). The [Blood] / [Vitreous] ratios (± SD) were 4.75 (±4.85), 35.71 (±23.28), 33.18 (±28.78), 1.56 (±0.77) for bromazepam, nordazepam, oxazepam, and meprobamate respectively.

The correlation between blood and vitreous concentrations is positive for the meprobamate in the studied population, which is not the case for the three studied benzodiazepines. The [Blood] / [Vitreous] ratio is much more important for the nordazepam and the oxazepam, than for the bromazepam

and the meprobamate. To explain this behavioral difference, further studies are necessary.

Benzodiazepine, Meprobamate, Vitreous

K37 Chemical Warfare Agent Decontamination Reactions in Ionic Liquids (I): Decontamination of Diisopropylfluorophosphate (Simulant for Sarin) and Bis(2-ethylhexyl) Phosphite (Simulant for VX) in DMPITf2N

John S. Wilkes, PhD, Joseph A. Levisky, MS, Adrian Hermosillo, BS, Patrick J. Castle, PhD, Cynthia A. Corley, BS, Sherri-Jean Adams, BS, Keith A. Sanders, BS, Ian S. Tuznik, BS, and Donald M. Bird, PhD, Department of Chemistry, Chemical Research Center, United States Air Force Academy, 2355 Fairchild Drive, Suite 2N225, Colorado Springs, CO 80840*

After attending this presentation, attendees will obtain an understanding of some of the basic research being conducted on decontaminating chemical warfare agents on buildings, vehicles, equipment, and personnel. The attendee will gain a better understanding of the role of ionic liquids as solvents, reactants, and catalysts in organic chemical reactions.

This presentation will impact the forensic sciences by acquainting first responders, medical examiners, coroners, investigators, and morgue attendants with some of the research efforts currently underway to neutralize facilities/equipment that may be exposed to various nerve agents. The impact of this paper is to instill confidence in all personnel that research is being conducted to establish protocols that minimize personal risks of exposure and rapidly return exposed equipment to use.

Because of 9/11, the vulnerability of America to attack became apparent and the threat of chemical attacks from within became real. Shortly after 9/11, major efforts were initiated to prepare for a chemical attack. The efforts involved establishing rapid and reliable decontamination processes for chemical warfare agents. In order for a decontamination process to be effective, it must be rapid, generate non-toxic reaction products, and be compatible with the environment. In studying decontaminating processes of chemical warfare agents, "simulants", in lieu of the actual chemical warfare agent itself, are used. Simulants are chemical compounds that are similar in chemical composition and physical properties to the actual agents, but considerably less toxic. For this study, the simulants, diisopropylfluorophosphate (DFP) and bis(2-ethylhexyl)phosphate (BEHP), were chosen which simulate the nerve agents Sarin and VX, respectively. The ionic liquid selected for study was 1,2-dimethyl-3-propyl imidazolium bistrifluoromethylsulfonyl amide ($DMPITf_2N$). $DMPITf_2N$ was selected because of its favorable hydrophilic/hydrophobic properties.

The objective of studying decontamination reactions in $DMPITf_2N$ as the ionic liquid is threefold: (1) identify those chemical compounds that are reactive with simulants in $DMPITf_2N$, (2) determine the composition of the reaction products, and (3) develop a reaction matrix that isolates the reactants and products from the environment.

In this report we describe the results of the reactions between DFP and BEHP with tetraalkylammonium hydroxide/methanol in DMPITf2N ionic liquid and the reaction between DFP and ethanolamine in $DMPITf_2N$. The reactions with tetraalkylammonium hydroxide/methanol were extremely rapid and produced both hydrolysis and alcoholysis reaction products. The reaction between DFP and ethanolamine resulted in nucleophillic substitution of the P-F bond with formation of isomeric phosphate esters and phosphoramides. A ^{1}H NMR Mercury 300 NMR was used to monitor the reaction between ethanolamine and DFP.

A liquid chromatograph coupled to an exact mass time-of-flight mass spectrometer (LC/MS-TOF) was used to identify the reaction products. A polar and aromatic reversed phase selectivity ether-linked phenyl with polar endcapping LC column (Synergi^{tm} Polar-RP^R) was used. A mobile phase gradient elution with methanol and 5mM ammonium formate from 30 – 95% over 12 minutes at 0.3 mL/minute flow provided good retention and resolution. Electrospray ionization was used as the ionization source. A discussion of the TOF fragmentation patterns of the reaction products is presented. The data presented here are results of ongoing research.

Chemical Warfare Agents, VX, Sarin

K38 Chemical Warfare Agent Decontamination Reactions in Ionic Liquids (II): Decontamination of Chloroethylethyl Sulfide, Simulant for Sulfur Mustard (HD), in DMPITf2N

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After attending this presentation, attendees will obtain a better understanding of some of the basic research associated with chemical warfare agent decontamination processes. The blister agent, chloroethylsulfide commonly referred to as mustard gas (HD), presents challenges to the scientific community in both its decontamination, if used as an offensive agent, as well as its disposal, as mandated by international agreements. The attendees will gain awareness of the chemistry being developed to convert this toxic material into non-toxic products by carrying out oxidation reactions in ionic liquids.

This presentation will impact the forensic sciences by acquainting first responders, medical examiners, coroners, investigators, and morgue attendants with some of the research efforts currently underway to neutralize facilities/equipment that may be exposed to chemical warfare agents. The impact of this paper is to instill confidence in all personnel that research is being conducted to establish protocols that minimize personal risks of exposure and at the same time will result in a rapid return of exposed equipment to use without fear of prolonged contamination or risk of exposure.

Many techniques currently exist, and more are being developed for the detection of toxins in chemical, biological and nuclear attacks. With the attacks of 9/11 and acts of terrorism abroad, the need has arisen for the scientific community to develop techniques centered upon a reactive posture. The end goal is to anticipate and mitigate the adverse effects of an actual chemical attack through a chemical process designed to be effective and rapid.

In this presentation we describe the results of some of the basic research being conducted in developing a reaction medium that: (a) will identify those chemical reagents that react with mustard gas stimulant (CEES) in an ionic liquid, (b) determine the composition of the reaction products, and finally (c) develop a reaction matrix that contains the reactants and products preventing them from entering the environment, i.e. Green Chemistry compatible. The ionic liquid selected for this study was 1,2-dimethyl-3-propylimidazolium bistrifluoromethylsulfonyl amide (DMPITf2N) because of its excellent hydrophillic and hydrophobic properties.

The copper II catalyzed oxidation of CEES with hydrogen peroxide in the DMPITf2N to the corresponding sulfoxide occurred with ease. Copper (II) bistrifluoromethylsulfonyl amide was selected as catalyst because of its compatability and solubility in DMPITf2N. A Mercury 300 NMR (^{1}H NMR) spectrometer provided a convenient method of monitoring the decrease in concentration of CEES in the ionic liquid. A liquid chromatograph coupled to an exact mass time-of-flight mass spectrometer (LC/MS-TOF) was used to identify the reaction products. A polar and aromatic reversed phase selectivity ether-linked phenyl with polar endcapping column (Synergi^{tm} Polar-RP^R) was used. A mobile phase gradient elution with methanol and 5mM ammonium formate from 30 – 95% over 12 minutes at 0.3 mL/minute flow provided good retention and resolution. Electrospray Ionization was used as the ionization source. A Thermo Electron Corporation PolarisQ ion trap GC/MS system was also used to determine the presence of remaining sulfide and sulfoxide and sulfone formation. A discussion of the TOF fragmentation patterns of the reaction products is presented. The data presented here are results of ongoing research.

Chemical Warfare Agents, Mustard Gas Simulant, Ionic Liquids

K39 Determination of Trace Levels of Benzodiazepine in Urine Using Capillary Electrochromatography – Time-of-Flight Mass Spectrometry

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The goal of this presentation is to present the benefit of using a monolith as a stationary phase in separation science and its hyphenation with a mass spectrometry detection.

This presentation will impact the forensic science community by providing information regarding the detection of trace level of benzodiazepines which are common drugs used as tools in drug facilitated sexual assault (DFSA).

Benzodiazepines are substances with a wide range of therapeutic uses; suitable for the treatment of sleeplessness, anxiety, increased muscle tone or epilepsy.

Mainly because they can produce anterograde amnesia, benzodiazepines are common drugs used as tools in drug facilitated sexual assault (DFSA). These drugs are comprised of a 1,4– diazepine ring with a benzene ring fused to carbons 6 and 7 and typically a phenyl group attached to carbon 5. Following an incident of DFSA, benzodiazepines may be present in very low concentrations. A successful analytical method for the analysis of these compounds may require detection limits below 10 ng/mL. Thus a highly sensitive analytical method is required.

This work details a method for the separation and determination of ten benzodiazepines in urine using capillary electrochromatography–time of flight mass spectrometry (CEC–MS(TOF)) and an hexyl acrylate-based porous monolith. The time of flight mass spectrometer proves to be able to determine exact mass of protonated benzodiazepines to three decimal places. This high selectivity along with the CEC separation, provides an effective method for the identification of benzodiazepines. Linearity is satisfactory for all compounds in the concentration range of 25–500 ng/mL for lorazepam and 12.5–500 ng/mL for the others. The relative standard deviations are between 1.4–2.3% for retention times and 1.1–9.2% for relative areas. Using the monolithic stationary phase, a pre-concentration step is achievable and permits a 75–140 fold improvement in sensitivity. This strategy allows the quantification of these drugs down to 1 ng/mL in urine. This method was used for the analysis of benzodiazepines in spiked urine samples.

Benzodiazepine, Electrochromatography, Mass Spectrometry

K40 Excretion of 11-Hydroxy-Δ^9-Tetrahydrocannabinol (11-OH-THC), and 11-nor-Δ^9-Tetrahydrocannabinol-9-Carboxylic Acid (THCCOOH) in Urine From Chronic Cannabis Users During Monitored Abstinence

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After attending this presentation, scientists will understand the urinary excretion of cannabinoids in chronic cannabis users, a population that is rarely studied due to the difficulty and cost of sequestering individuals for extended periods of time.

This presentation will impact the forensic science community by demonstrating how the urinary 11-OH-THC excretion data conducted with heavy chronic daily cannabis users during monitored abstinence clearly indicate that 11-OH-THC in urine cannot be used to indicate recent cannabis use.

Seven healthy participants (aged 20-35, four males & three females), who self-reported an extended history of daily cannabis use, provided written informed consent for this IRB-approved study. Subjects self-reported chronic daily smoking of between one and five cannabis "blunts" prior to entering the closed research unit. During the study, all subjects were under continuous medical surveillance for up to 29 days at the NIDA Intramural Research Program to prevent self-administration of additional drugs. Each urine specimen (n = 259) was collected individually *ad libidum*. Two mL urine specimens were hydrolyzed by a tandem enzyme (*E. coli β*-glucuronidase)/alkaline method, extracted by SPE (Clean Screen® ZSTHC020 extraction columns, United Chemical Technologies, Bristol, PA), and derivatized with BSTFA for 30 min at 85°C. Trimethylsilyl derivatives of 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC), and 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THCCOOH) were resolved and quantified in a 2-dimensional/cryofocusing chromatography system (Agilent 6890 GC/5973MSD) operated in electron impact selected ion monitoring (EI/SIM) mode. Limit of quantification (LOQ) was 2.5 ng/mL for both analytes. Accuracy of the method ranged from 87.6% to 102.1%. Intra- and inter-assay precision, as percent relative standard deviation, were less than 8.6% for both analytes.

Time of last detection (> LOQ) of 11-OH-THC for all subjects in urine ranged from 180 – 716 hours (7.5 to 29.8 days). 11-OH-THC maximum concentrations ranged from 25 – 133 ng/mL (mean 79.7 ± 40.1, median 67 ng/mL). Maximum concentrations of THCCOOH ranged from 117 – 766 ng/mL (mean 455.3 ± 208.3, median 482 ng/mL). All participants also had THCCOOH positive urine specimens at the LOQ on the last day of residence between 7.5 to 29.8 days. It also is important to evaluate urinary THCCOOH concentrations at the 15 ng/mL federally mandated cut off utilized by most laboratories. Employing the 15 ng/mL cutoff, THCCOOH urine specimens also were positive throughout residence on the research unit for 7.5 to 29 days.

These data indicate that following chronic cannabis smoking, 11-OH-THC can be measured in urine for up to 29 days, negating its value as a urinary biomarker of recent cannabis use.

Cannabinoids, Urine, GC/MS

K41 Gamma-Hydroxybutyrate (GHB) in Saliva: A GC/MS Method Applicable to Toxicological and Physical Evidence

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The goal of this presentation is to introduce attendees to the potential use of saliva as an alternative biological matrix and as a tool in GHB screening analysis and to establish GC/MS as a sensitive analytical technique for the detection of GHB in saliva.

This presentation will impact the forensic science community by describing a proposed method for the rapid, selective and accurate toxicological screening of saliva analysis for forensic purposes. The use of a surrogate standard provides a quantitative measure of extraction and preparation efficiency that is matrix specific. The method described here could be applied to swabs, neat saliva, and possibly physical evidence such as saliva on drink glasses. Current research is focused on the latter application.

GHB and related compounds have been known for years because of their illicit use in drug facilitated sexual assault (DFSA) and to a lesser extent, as party drugs. This problem is exacerbated by GHB's rapid clearance rate and short half life of ~30 min. For this reason, it would be useful to develop a rapid screening analysis from a biological matrix that predictably tracks

plasma drug concentrations. Oral fluids, which can be collected non-invasively, are an attractive option. Unfortunately, saliva drug concentrations are generally significantly lower than those in urine, which creates challenges for method development.

A sensitive and specific gas chromatography-mass spectrometer (GC-MS) method has been developed using selective ion monitoring (SIM) for the identification and quantification of gamma-hydroxybutyric acid (GHB) in saliva. In this approach, 1.0 µl of synthetic saliva was spiked with 1.0 µl of GHB-d6 as the internal standard. As an added quality assurance method, 1.0 µl of 1,7-heptanediol is added to all samples as a surrogate spike. The purpose of the surrogate is to track the efficiency of extraction and preparation procedures.

After a silyl-derivatization the sample was injected at a split ratio of 10:1. The following ions were monitoring: GHB 233, 234; GHB-d6: 239, 240, 241; 1,7-heptanediol: 55, 73, 97. No interferent peaks were observed. The LOQ was determined to be 0.5 ppm with a linear dynamic range of 0.5 ppm to 50 ppm. Quality-control samples (5 ppm, 20 ppm, 30 ppm) were prepared for evaluation of analytical precision. Variation was found to be from 1.07 to 9.44% in both intra-day and day-to -day experiments respectively. Surrogate recovery from saliva samples fell in the range of 94.6 to 100% with an average of 98.37% and a corresponding % RSD of 1.2%. Data obtained from validation were compared with results from sample prepared drying saliva before the derivatization process. Blank samples from lab staff were analyzed to estimate endogenous GHB in saliva. Values in the range of 2-3 ppm were typical. These results will be presented.

The success of this method suggests a novel extension to physical evidence. Victims of sexual assault may leave biological evidence such as saliva on surfaces like the exterior of glasses, tissues, and cigarettes. If the saliva was deposited after the illicit drugging occurred, the deposited saliva may provide valuable investigative information and evidence of a drug-facilitated assault. With drugs such as GHB that have rapid clearance rates, the capability to detect elevated GHB in deposited saliva samples could be significant.

In the present work, we utilized the GC/MS method for a screening test for GHB in saliva on objects such as cigarettes, bottles, cups, plastic glasses. Blank saliva was spiked with GHB at different concentrations. 5 µl of those samples were then spread out on the surfaces. At time intervals, saliva samples were extracted from the objects with a swab saturated with methanol. After a centrifugation, the supernatant was dried and reconstituted in 500uL of methanol, of which 1 uL was injected into the instrument. This methodology precludes quantitation, but does afford reliable qualitative results at low concentrations.

Gamma-Hydroxybutyric acid (GHB), GC-MS, Saliva

K42 Methamphetamine Involved MVA Fatalities in Phoenix: A Seven Year Postmortem Study

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After attending this presentation, attendees will learn about the fatality statistics associated with motor vehicle accidents (MVAs) involving operators driving under the influence of methamphetamine in the Phoenix Metropolitan area for the past seven years.

This presentation will impact the forensic community and/or humanity by increasing awareness not only in Arizona but throughout the United States concerning the growing problem of driving under the influence of drugs, in particular methamphetamine.

The Maricopa County Office of the Medical Examiner (OME) provides medicolegal investigations into all deaths in a population of 3.7 million to determine the cause and manner of death. In this study, the 31,274 cases admitted to the office between 2000 and 2006 were examined, and of those cases, 2,449 were ruled MVAs. Of those 2,449 MVAs, 168 of them tested positive for methamphetamine. It was reported by OME Investigators and various law enforcement agencies that the driving behavior of these individuals included speeding (16), running a red light (14), collisions caused by either crossing the center line of traffic (31) or rear-ending another vehicle (10) or other various means (32), single vehicle accidents caused by leaving the roadway (21) and roll-overs (27), as well as other erratic driving behavior (17).

In each case, a blood sample was screened using ELISA for the presence of methamphetamine with a 0.05 mg/L cutoff value. Each sample that screened positive was then extracted by liquid-liquid extraction using d10-amphetamine and d11-methamphetamine as internal standards followed by pentafluoropropionic anhydride derivatization. This extract was then analyzed quantitatively using selected ion monitoring (SIM) mode with gas chromatography/mass selective detection (GC/MSD) using electron impact ionization with a limit of detection of 0.01 mg/L and a limit of quantification of 0.025 mg/L. The methamphetamine results for the 168 methamphetamine involved MVAs ranged from 0.025 mg/L to 11.34 mg/L, with an average of 1.05 mg/L and a median of 0.53 mg/L. Of these cases, 117 of them (70%) were above the suggested therapeutic value of 0.20 mg/L with 19 of those (11%) above 2.5 mg/L. The amphetamine results ranged from 0.025 mg/L to 1.16 mg/L, with an average of 0.11 mg/L and a median of 0.08 mg/L.

When this data is broken down by year, it shows a trend of increasing numbers of methamphetamine involved MVAs each year from 2000-2004, with a plateau since 2004 despite yearly increases in county population, OME admitted cases, and total MVA cases. Because methamphetamine is currently the most frequently encountered clandestinely produced drug in the United States, various federal and state laws have recently been passed that place restrictions on the sale of methamphetamine precursors, and that increase the consequences faced by methamphetamine offenders. In the state of Arizona, there have been various government and private sponsored programs to combat the growing problem of illicit methamphetamine use. There is some hope that these laws and organizations will help to curb the methamphetamine use in Maricopa County; ultimately decreasing the number of methamphetamine involved MVA fatalities the office receives each year.

Data concerning 2007 methamphetamine involved MVAs is currently being collected and will be added to the current data, and presented along with the seven years represented so far.

Methamphetamine, MVA, Fatalities

K43 "Less Than Perfect" DUI Drug Cases: Do You Think They Were Impaired?

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After attending this presentation, attendees will be given the opportunity to discuss the pros and cons of using only limited information to establish suspected Driving Under the Influence of Drugs (DUID).

This presentation will impact the forensic science community by assisting toxicologists who support Drug Recognition Expert (DRE) programs as well as those who regularly testify on Driving Under the Influence of Drugs cases for both the prosecution and defence.

Developing forensic opinions on persons suspected of drug-impaired driving is regularly performed by forensic toxicologists who testify in court. Accomplishing this based solely on records review can be difficult under the best circumstances. The widely used "triad of results" involves: 1) consideration of the nature of any accident and events which led up to it, including observed driving behaviors, 2) field sobriety tests (FST's) and/or drug recognition examinations (DRE's) by law enforcement, and 3) qualitative and quantitative toxicology findings, preferably from a blood sample. But can a valid opinion on impairment be developed with less-than-

perfect information? You be the judge!

Part One: The "Hit-and-Run Nanny": The defendant in the first case, a resident alien who had worked for several years as a nanny, was driving to work when she drifted off the road, over a curb and struck and killed two young children out for a walk with their mother. Afterwards, the suspect fled on foot and was apprehended after two days. Blood collected at the time of her arrest tested negative for drugs and alcohol.

The information available for case review included accounts of the suspect's criminal background, personal history, behavior on the accident day, her witnessed driving pattern prior to the accident and behaviors immediately afterwards, and lastly, the defendant's admissions about her drug consumption before the accident. When the accident occurred, she was driving on a suspended license and had four previous DUI alcohol convictions. She had had marital problems with a recent separation from her husband due to heavy drinking. She had prescriptions for Vicodin® (hydrocodone) and Flexeril® (cyclobenzaprine). She had apparently taken 110 Vicodin pills in the prior 22 days and had prescriptions from several physicians. Neighbors reported seeing her drinking alcohol earlier that afternoon.

Observed driving patterns and behaviors included weaving, jackrabbit starts and stops, sleepy and confused appearance, and driving with her hair covering her face. She admitted ingesting four or more pills of each medication that day. What is your opinion? Was her driving was impaired by drugs and perhaps alcohol when the accident occurred?

Part Two: Can a Positive Urine THC Metabolite (THC-COOH) Be Used to Prove Impairment in a Driving Under the Influence (DUID) - Cannabis Case?: A suspect was stopped for speeding at midnight on Colorado Interstate 70, a section of the interstate with a steep 6% grade. According to the arresting officer the subject "displayed indicators of being under the influence of alcohol or drugs or both". Officer observations included "speech was slow and thick tongued and that he had a brown-green coating on his tongue". The subject admitted taking a Vicodin® pill earlier that day. His hand was in a cast. He agreed to perform standardized field sobriety tests (SFST's) and according to the officer, he failed them. A search of the subject's vehicle revealed an open 24-pack of beer, an open can in the console, and a glass pipe. The subject was arrested at 0020 hours and agreed to take a breath alcohol test (which was <0.05%) and a urine sample was also collected.

The urine screened positive for cannabinoids and confirmed for delta-9-THC-COOH (84 ng/ml). The sample also screened positive for opiates and confirmed for hydrocodone. The subject apparently had spent the day with friends, and reportedly consumed a minimal amount of beer and no other drugs within six hours of being arrested. He reported recent dental surgery and was taking Vicodin received from his dentist for pain.

The officer who conducted the SFST's was not a certified Drug Recognition Expert (DRE). Based upon the low breath alcohol concentration, there was no charge of driving while intoxicated with alcohol.

You decide. Do the positive drug and drug metabolite findings in the subjects urine substantiate, along with his failure to pass the SFST's, that he was impaired by drugs to such a degree at the time of driving that he should be charged with DUID? If so, what was the contribution of cannabinoids? Of hydrocodone? Of alcohol?

Drugs and Driving, Forensic Toxicology, Impairment

K44 Driving Under the Influence of Cannabis: Are Science-Based Concentration Limits for Tetrahydrocannabinol (THC) in Blood Practical to Enforce?

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After attending this presentation, attendees will acquire up-to-date information about various forensic aspects of driving under the influence of cannabis.

This presentation will impact the forensic science community by providing information on the effectiveness of zero tolerance legislation as a practical and pragmatic way to simplify the prosecution of offenders.

Those attending this presentation will acquire up-to-date information about various forensic aspects of driving under the influence of cannabis. The age and gender of offenders are reported in relation to the concentrations of tetrahydrocannabinol (THC) in blood of motorists apprehended over a 10-year period. Results are compared from before and after a zero-tolerance law for driving under the influence of drugs (DUID) was introduced in Sweden. The forensic community in North America will learn about the effectiveness of zero tolerance legislation as a practical and pragmatic way to simplify the prosecution of offenders.

Although cannabis and its various preparations are considered illicit drugs in most countries, these psychoactive substances are widely used for recreational purposes and as such represent a problem for traffic safety. Some countries have a fairly liberal attitude towards possession of cannabis for personal use, whereas in other nations, this constitutes a criminal offence. Accordingly, there is much ambivalence about the danger of cannabis use and abuse in society and re-classification as a scheduled substance is sometimes considered. Indeed, there is increasing discussion and debate among scientists and politicians about the pros and cons of cannabis as a recreational drug and the legal prescribing of cannabinoids for treatment of certain medical conditions.

The pharmacologically active constituent of cannabis, hashish and marijuana is Δ^9-tetrahydrocannabinol (THC), which displays a complex pharmacokinetic profile owing to its high lipid solubility, protein binding and large distribution volume. The forensic evidence necessary to verify that a person has taken cannabis comes from finding THC or its main metabolites (6-hydroxy-THC and carboxy-THC) in blood, urine or other body fluids. Knowledge about the concentration of THC in blood is necessary to permit drawing conclusion about the effects on a person's performance and behavior and the likelihood of drug-related impairment and the risk of a traffic crash. In our laboratory THC is determined in blood samples by gas chromatography-mass spectrometry with deuterium labeled internal standards (d_3-THC). The limit of quantitation (LOQ) of this method in routine use is 0.0003 mg/L (0.3 ng/mL).

Enforcement of laws pertaining to driving under the influence of drugs (DUID) other than alcohol are either structured around measuring drug-related effects on the individual concerned or some threshold concentration in blood is set by statute, above which a person is liable to prosecution. The creation of zero-.tolerance laws is increasingly favored in European nations for illicit drugs so that any measurable amount in a specimen of blood constitutes an offence of impaired driving. The presence of such a drug or its metabolites in urine but not in blood does not motivate charging a person with DUID in European nations.

Considerable interest exists in trying to establish so-called "science based" concentration limits for driving under the influence of cannabis. The scientific background for this stems from measurement of cognitive and psychomotor impairment after smoking marijuana, clinical correlates of THC concentrations in blood, epidemiological surveys of cannabis-related traffic crashes and also a limited number of on-the-road driving performance tests. Roadside surveys of the risk of a crash as a function of the blood alcohol concentration exist (e.g., the Grand Rapids study) but equivalent studies for cannabis are lacking. The threshold concentration limit of THC in blood under such a *per se* statute has not yet decided but this will most likely be set fairly high at 0.002-0.003 mg/L (2-3 ng/mL) or even higher.

Over a 10-year period between 18% and 30% of all DUID suspects apprehended in Sweden had measurable amounts of THC in their blood (> 0.0003 mg/L) either alone or together with other drugs. The mean age (± SD) of cannabis users was 32.6 ± 9.4 y (range 15-66 y) with a strong predominance of men (94%). The frequency distribution of the concentrations of THC in blood (N = 8,803) was markedly skewed to the right with mean, median and highest values of 0.0021 mg/L, 0.0010 mg/L and 0.067 mg/L, respectively. The concentrations of THC were less than 0.001 mg/L in 42% of cases and below 0.002 mg/L in 60% of cases. No statistically

significant correlation existed between the concentration of THC in blood and the person's age (r = -0.027). THC concentrations in blood were higher when this was the only psychoactive substances present (N = 1,281); mean 0.0036 mg/L, median 0.002 mg/L and 26% were below 0.001 mg/L and 40% were now less than 0.002 mg/L. The concentrations of THC in blood were similar in a population of users of illicit drugs (non-traffic cases). Based on studies from Sweden it can be shown that at least 40% of drivers abusing cannabis would evade prosecution if the THC limit in blood was set at 0.002 mg/L (2 ng/mL).

The complex kinetics of THC means that the concentrations in blood at the time of driving are likely to be considerably greater than at the time of sampling blood, which occurs about 30-90 min afterwards, owing to movement of the active substance (THC) from the central blood into peripheral tissues and lipid compartments. The notion of establishing a science-based concentration limit for THC in blood (e.g., 0.002-0.003 mg/L) or higher, as being discussed by some investigators, would mean that many individuals who had smoked marijuana before driving would evade prosecution. Zero-tolerance or LOQ laws are a much more pragmatic way to enforce DUID legislation.

Cannabis, Drugs, Driving

K45 Instances of Marijuana, Driving, Blood Concentrations, Field Sobriety Tests, and Prediction Models

Joseph J. Saady, PhD,and Terri H. Woods, BS, Virginia Department of Forensic Science, 700 North 5th Street, Richmond, VA 23219*

After attending this presentation, attendees will understand some of the difficulties in the correlation of driving related behaviors and blood concentrations of marijuana.

This presentation will impact the forensic community by analyzing various aspects of marijuana and driving, to include the blood concentration, Field Sobriety Test results, the analytical analysis and the timing and manner of the last smoke.

One of the very difficult court testimonies for a forensic toxicologist pertains to marijuana, which is the most frequently encountered drug in the Virginia DUID program, with the exception of ethanol. Some of the issues for consideration in the interpretation of results involve the time of last smoke, the rapid elimination of tetrahydrocannabinol (THC) from the blood, the blood collection time following the incident, the analytical testing and the duration of storage of the blood sample, and physiological effects as close to the time of a suspected driving incident as possible.

The authors will review Virginia DUID cases where there was some reasonable suspicion that smoking occurred close to or at the time of the police stop, as noted by law enforcement personnel. The collection of the blood specimen occurred from one to four hours after the stop. The laboratory typically receives the blood specimen by mail within one week of collection, and stores it refrigerated until analysis. Samples are analyzed by a modified Kemp et.al. method, usually within six weeks of receipt. Briefly, two mL of blood is mixed with THC-d3 and THCA-d3 internal standards, vortexed while adding cold acetonitrile and refrigerated until the phases separate. Acetonitrile is back extracted with 0.2 N NaOH into hexane:ethyl acetate (9:1), evaporated under nitrogen and derivatized with trifluoroacetic acid anhydride., heated, evaporated and reconstituted with heptane, transferred to an autosampler vial for gas chromatography mass spectrometry (GC/MS) for THC selected ion monitoring (SIM) quantitation. The saved NaOH fraction is acidified with 1 N HCl, extracted with hexane:ethyl acetate (9:1), evaporated under nitrogen and derivatized with BSTFA with 1% TMCS, transferred to an autosampler vial for gas chromatography mass spectrometry (GC/MS) for tetrahydrocannabinol carboxylic acid (THCA) selected ion monitoring (SIM) quantitation.

Field sobriety tests (FST) generally consisted of recitation of some portion of the alphabet or counting, Horizontal Gaze Nystagmus (HGN), Walk and Turn and One Leg Stand. Most cases showed a number of errors at nearly all concentrations.

Based on statements by law enforcement, predictive modeling (Huestis Model II) produced reasonable estimates of the smoking time using the average, in most of the cases. In some instances the trooper saw the disposal of the cigarette, or could see smoke in the vehicle. In other instances, statements by the suspect led to the conclusion that smoking occurred recently (e.g., "I smoked marijuana at a friends house 15 or 20 minutes ago").

In conclusion, blood THC concentrations ranged from 1 to 27.5 ng/mL, while (THCA) ranged from 2 to 343.5 ng/mL. There was a relatively poor correlation between THC concentrations and FST errors. However, there was a good correlation between errors on the FST and the presence of THC in the blood. Using information provided by law enforcement concerning the time of last smoking, Model II produced reasonable estimates.

Marijuana, DUID, Concentrations

K46 Plasma Cannabinoid Concentrations in Daily Cannabis Users During Seven Days of Monitored Abstinence

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After attending this presentation, attendees will learn that Δ^9-tetrahydrocannabinol (THC) plasma concentrations can exceed 0.25 ng/mL for more than seven days during monitored cannabis abstinence in daily cannabis users.

This presentation will impact the forensic community by influencing interpretation of plasma THC concentrations from daily cannabis users.

In the presence of corroborating evidence, detection of THC in whole blood at ≥ 2 ng/mL is commonly considered consistent with recent cannabis use in driving under the influence of drugs (DUID) cases and other forensic investigations. This laboratory has previously reported detectable THC in plasma (≥ 0.5 ng/mL) 3 to 27 hr after smoking a single 1.75 or 3.55% THC cigarette, while the inactive metabolite, 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THCCOOH) was detected at 0.5 ng/mL for 48 to > 168 hr. Few data are available on analyte detection in plasma of daily cannabis users during periods of monitored abstinence.

Twenty-eight, self-reported daily cannabis users (ages 19-36, 46.4% male, 85.7% African American) provided written informed consent for this IRB-approved study, where they resided in a closed clinical research unit for 7 days. Plasma specimens were collected upon admission and once every 24 hr thereafter. Cannabinoids were extracted by solid phase extraction (SPE) using ZSTHC020 columns (United Chemical Technologies, Inc., Bristol, PA) and derivatized with *N*,*O*-bis-(trimethylsilyl)trifluoroacetamide + 1% trimethyl-chlorosilane (BSTFA + 1% TMCS). Derivatized extracts were injected into an Agilent 6890 gas chromatograph (GC)/5973 mass selective detector (MSD) system operated in electron impact (EI)/selected ion monitoring (SIM) mode. A two dimensional GC method with cryofocusing was developed and validated for the quantification of THC, 11-OH-THC, and THCCOOH. Split calibration curves (low, 0.125 – 25 and high, 25 – 100 ng/mL) were constructed with $r^2 > 0.99$. Limits of quantification (LOQ) were 0.25 ng/mL for all analytes.

After more than 16 hr of monitored abstinence, 92.6% of participant plasma specimens (N = 27) were positive for THC (≥ 0.25 ng/mL). On Days 3, 4, 5, 6, and 7, 84.6 (N = 26), 79.2 (N = 24), 70.8 (N = 24), 66.7 (N = 24), and 76.0% (N = 25) of participant plasma specimens contained detectable THC, respectively. Not all participants had adequate specimen volume on all days.

Of the 19 participants' plasma specimens testing positive for THC on day 7 (≥ 0.25 ng/mL), 9 tested positive with an LOQ of 1 ng/mL, while 4 were positive using a 2 ng/mL LOQ.

Sixteen participants' plasma specimens had detectable THC on days 2,

4, and 7; median concentrations were 1.6 (range 0.8 - 7.3), 1.4 (range 0.5 - 7.5), and 1.2 (range 0.3 - 5.5) ng/mL, respectively. Fewer specimens were positive for 11-OH-THC; median concentrations were 2.4 (N = 3, range 2.1 – 3.3), 1.2 (N = 2, range 0.73 – 1.75) ng/mL, and not detected (N = 16) on days 2, 4, and 7, respectively. Median THCCOOH concentrations in these 16 participants' specimens were 25.9 (range 7.2 – 189.4), 19.4 (range 4.3 – 88.3), and 11.5 (range 2.8 – 45.6) ng/mL, on days 2, 4 and 7, respectively.

Interpretation of plasma and whole blood cannabinoid concentrations is important in DUID and other forensic cases. For the first time, we present evidence of the presence of THC in plasma for multiple days during monitored abstinence, suggesting that its detection in plasma may not indicate recent use in individuals consuming cannabis on a daily basis. Bioaccumulation of THC in deep tissue compartments and gradual release from tissue stores into the bloodstream during cannabis abstinence may explain this prolonged seven day THC detection window.

THC, Plasma, Cannabis

K47 The Z Drugs: An Update for Forensic Toxicologists in Light of DUID Cases

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After attending this presentation participants will have a greater understanding of the Z drugs, how they produce the effects commonly seen in DUID cases and metabolism and excretion profiles that affect the forensic toxicologist abilities to detect the drug or metabolites. In addition, recent pharmacological research will be summarized concerning such issues as sleep driving and other aberrant behavior.

This presentation will influence the forensic science community who support suspected DUID and drug facilitated sexual assault cases by enhancing their understanding of the drug mechanisms and current challenges to interpretive issues.

The "Z-drugs" are non-benzodiazepine sedative hypnotic available in standard release and extended release formulations. Zolpidem (Ambien) has consistently finished in the "Top 20" of the 200 most prescribed medications over the last seven years. Zolpidem is commonly prescribed for treatment of insomnia. Lunesta (Eszopiclone) has been approved by the U.S. Food and Drug Administration for long term treatment of insomnia since 2004. Eszopiclone is a nonbenzodiazepine hypnotic that is a pyrrolopyrazine derivative and is a steroisomer of zopiclone (Imovane, Noctitrex, Ximovane, Zimovane), which is not currently available in the U.S. Zaleplon (Sonata) is also a nonbenzodiazepine hypnotic from the pyrazolopyrimidine class. Zaleplon interacts with the GABA receptor complex and shares some of the pharmacological properties of the benzodiazepines. Although not a benzodiazepine, zaleplon can cause similar effects: anterograde amnesia (forgetting the period during the effects) as the most common side effect.

Multiple cases will be presented highlighting some of the analytical and interpretation challenges presented by the "Z-drugs". Zolpidem blood concentrations in a few selected cases ranged from 190 to greater than 4,000 ng/mL. Clearly some of these drivers' blood concentrations dramatically exceed those expected from single oral dosing for night time hypnotic effect. A typical case of Zolpidem impaired driving is presented: A law enforcement officer observed a subject crash into the rear of a parked car. The officer also noted "bizarre driving" with the subject driving in reverse for one block, then stopping in the line of traffic for one minute (one vehicle had to swerve to avoid crash). The officer pulled up behind & activated emergency lights; however the subject didn't notice the officer and started driving forward. Eventually the subject stopped. The subject was wearing a fur lined winter cap over a baseball cap and sunglasses over the top of prescription eyeglasses even though it was night time at the time of the incident. The subject exhibited delayed responses to the officer's questions, slow slurred speech and seemed confused. The subject was unsteady and needed to brace on the car to attempt Standardize Field Sobriety Tests (SFST). The subject exhibited multiple clues on all 4 SFSTs. The subject was arrested and taken in for a blood sample. Throughout the examination the subject was unable to recall any of the recent incidents. The subject stated: "I'm confused, lost and out of it". Toxicological analysis of the blood revealed zolpidem at 500 ng/mL and less than 50 ng/mL of citalopram.

As this case report demonstrates, "Z-drugs" have the potential to significantly impair the driving abilities of an individual. Dissemination of toxicological findings for cases such as these will assist forensic toxicologists in their own case interpretations.

DUID, Zolpidem, Zaleplon and Zopiclone

K48 Medical Devices and Their Impact on Death Investigations

Alberto Gutierrez, PhD, Office of In Vitro Diagnostic Device Evaluation and Safety, 9200 Corporate Boulevard, Rockville, MD 20850; Alphonse Poklis, PhD*, Medical College of Virginia, Box 98-165, Virginia Commonwealth University/Medical College of Virginia Hospitals Station, Richmond, VA 23298; Joseph A. Prahlow, MD*, South Bend Medical Foundation, 530 North Lafayette Boulevard, South Bend, IN 46601;and Ruth E. Winecker, PhD*, Office Chief Medical Examiner, Campus Box 7580, Chapel Hill, NC 27599-7580*

After attending these presentations participants will understand how medical devices such as blood glucose monitors, insulin pumps, patient controlled analgesia, intrathecal pumps, and defibrillators can impact death investigation by providing information about the events surrounding a death.

The presentation will impact the forensic community by providing information about medical devices, their evaluation, and assignment of cause and manner of death.

Introduction: There are a variety of medical conditions in which medical devices including blood glucose monitors, insulin pumps, patient controlled analgesia, intrathecal pumps, and defibrillators are employed and these devices are encountered with increasing frequency in forensic death investigations. Questions concerning the proper operation and potential tampering of these devices as well as historical information contained in them is of concern to a variety of forensic professionals.

Topics Covered: This special session will cover regulatory, pathological, toxicological, and safety issues related to medical devices.

A historical overview of these devices, their in vitro diagnostic evaluation and safety by the Food and Drug Administrations Center for Devices and Radiological Health (CDRH) as well as basic information on device regulation will be discussed. More than 20,000 companies worldwide produce over 80,000 brands and models of medical devices for the U.S. market. These devices rang from contact lenses and blood sugar monitors to implanted hip joints and heart valves. The CDRH makes sure that new medical devices are safe and effective before they are marketed. The center is also responsible for monitoring these devices throughout the product life cycle, collecting, analyzing, and acting on information about injuries and other experiences in the use of medical devices and radiation-emitting electronic products, setting and enforcing good manufacturing practice regulations and performance standards for medical devices, monitoring compliance and surveillance programs for medical devices.

A synopsis of techniques that might be used during autopsy when encountering an in vitro device as well as case studies in which the interaction of pathology and these devices played a role in the death will be included. Special procedures used during and following an autopsy can help with a diagnosis of device performance. These procedures can help when deciding on a cause and manner of death.

Toxicological case studies focused mainly on chronic pain treatment involving fentanyl patches and continuous analgesia infusion devices will be discussed. Aggressive treatment of chronic pain in recent years has lead

to an increase in the frequency of cases in which analgesic devices such as fentanyl patches and intrathecal pumps are encountered by forensic professionals. Investigations into the performance of these devices are often requested and the toxicology laboratory is faced with the task of testing the devices and interpreting the results. The case studies will be used to illustrate salient points such as proper storage of the devices, sampling and caution in interpretation.

Finally the session will conclude with a discussion of medical devices related specifically to the treatment of diabetes mellitus and case studies in which these devices played a role in deciding the cause and manner of death. In postmortem death investigation of deaths involving diabetes mellitus, having a record of recent blood glucose measurements can help in determining the level of control the decedent had prior to death and whether or not there were recent difficulties such as abnormally low or high blood glucoses. Diabetic deaths to nonketotic hyperosmolar coma and diabetic ketoacidosis can be diagnosed by high vitreous glucose, disturbances in vitreous electrolytes and the presence of acetone. However, deaths due to insulin overdose are usually a diagnosis of exclusion as methods for measuring postmortem insulin concentrations are not readily available or even reliable. Both traditional blood glucose devices and the new continuous blood glucose monitors can be accessed to provide historical information to aid in this diagnosis. Many insulin pumps also keep a continuous record documenting 7-14 days worth of blood glucose levels, insulin boluses (insulin given in response to a meal, snack or correction of high blood glucose), and total insulin use per day. Instruction on how to access the data these devices contain will be provided.

Medical Devices, Safety, Evaluation

K49 Methadone Disposition in Human Breastmilk and Plasma in the Immediate Perinatal Period

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After attending this presentation, attendees will learn about the methadone content in human breastmilk in the immediate postnatal period, its relationship to maternal methadone dose and maternal and infant plasma concentrations, and the variability in breastmilk methadone concentrations in fore- and hindmilk.

This presentation will impact the forensic science community by demonstrating how the findings that maternal methadone dose was unrelated to plasma and breast milk methadone concentrations and that infant methadone exposure from breastmilk was low, support the recommendation that methadone-maintained women be permitted to breastfeed their infants if appropriate and desired.

Methadone maintenance is the only recognized pharmacologic treatment for opiate dependency during pregnancy in the U.S. It is well established that breastfeeding is the optimal way to nourish an infant. Breastmilk confers known advantages to mother and infant and could be beneficial for methadone-exposed infants at risk for morbidity in the perinatal period. However, lactation among methadone-maintained women is frequently challenged due to lack of knowledge about this practice. 2,257 women enrolled in a comprehensive substance abuse treatment program for pregnant and post-partum drug dependent women were screened for participation. Any woman considered intoxicated or having a positive urine test was excluded from further participation. Eight methadone-maintained (dose range 50–105 mg/day), lactating women provided blood and breastmilk specimens on days 1, 2, 3, 4, 14, and 30 after delivery at the times of trough (just before methadone dose) and peak (3 hours after dosing) maternal methadone levels. Paired specimens of foremilk and hindmilk were obtained at each sampling time. Eight matched formula-feeding subjects had blood drawn the same days. Infant blood for both groups was obtained on day 14 concurrent with a heelstick for routine pediatric care. All infants underwent neurobehavioral testing using the NICU Network Neurobehavioral Scale on days 3, 14, and 30.

Breast milk was collected in polypropylene storage vials and stored at -20°C until time of analysis. Specimens were analyzed using a validated liquid chromatography atmospheric pressure chemical ionization tandem mass spectrometry method. Breastmilk, 0.5 mL was analyzed following protein precipitation and solid phase extraction. The limit of quantification (LOQ) was 10 ng/mL with a linear dynamic range of 10 – 500 ng/mL. Extraction efficiency was greater than 97% with inter- and intra-day imprecision < 20%. Blood was collected in heparinized tubes, centrifuged, and plasma separated and stored at -20°C until time of analysis. Plasma specimens were analyzed by gas chromatography mass spectrometry following solid phase extraction. LOQ for methadone was 5.0 ng/mL and range of linearity was 5 – 2000 ng/mL. Intra- and inter-day imprecision was <20%.

Repeated measures linear regression was used to determine whether there was a significant change over time days 1 through 30 in breastmilk methadone concentrations for each sampling time (trough prefeed, trough postfeed, peak prefeed, peak postfeed) and whether there was an effect of breastfeeding (yes/no), time (day 3, 14, 30) or breastfeeding by time interaction for neurobehavioral outcomes. Statistical significance was set at $P < 0.05$ for all analyses.

Methadone doses among subjects and controls varied little in the postpartum period and were (median (range): 70 mg (50 – 105 mg) at delivery, and days 14 and 30. Concentrations of methadone in breastmilk were low (range 21.0–462.0 ng/mL) and not related to maternal dose. There was a significant increase in methadone concentrations in breastmilk over time, and concentrations increased from pre- to postfeed in all cases aside from the first collection (colostrum). There were no significant effects of breastfeeding on neurobehavioral outcomes. Fewer infants in the breastfed group required pharmacotherapy for neonatal abstinence syndrome, but this was not a statistically significant finding. Infant plasma methadone concentrations obtained on day 14 of life were low, uniformly detected among all samples, and were unrelated to maternal methadone dose, maternal plasma methadone concentrations, and breastfeeding. Further, infant plasma methadone concentration was not related to the infant's need for pharmacotherapy for NAS or NAS scores. This research demonstrated that concentrations of methadone in breastmilk, even at peak maternal plasma methadone levels, are low in the perinatal period.

Methadone; Plasma, Breast Milk

K50 Disposition of Buprenorphine and Norbuprenorphine in Human Meconium

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This presentation will provide attendees with the first data on concentrations, ratios and extent of glucuronidation of buprenorphine and norbuprenorphine in meconium following controlled buprenorphine administration to a cohort of pregnant women.

This presentation will impact the forensic community by providing the first data on buprenorphine and norbuprenorphine excretion in meconium following controlled drug administration to a cohort of pregnant women. These data provide valuable information on buprenorphine and norbuprenorphine concentrations and the extent to which these analytes may be conjugated in meconium. Additionally, these data allow for correlations to be examined between maternal dose and meconium concentrations and between meconium concentrations and neonatal outcomes, providing critical information for clinicians.

Buprenorphine is currently being investigated in the United States as a pharmacotherapy for treating opioid dependence in pregnant women. The disposition of buprenorphine and norbuprenorphine was evaluated in meconium from infants born to nine women participating in a study approved by the Johns Hopkins Bayview Medical Center and National Institute on Drug Abuse Institutional Review Boards comparing methadone and buprenorphine for the treatment of opioid dependence during gestation.

Women were treated with 14-24 mg/day buprenorphine for the last 12-22 weeks of pregnancy. Meconium specimens (N=10, one set of twins) were analyzed using the first validated liquid chromatography-tandem mass spectrometry with atmospheric pressure chemical ionization method. Two aliquots (0.25 ± 0.1 g) of each specimen were analyzed, one with and one without enzyme hydrolysis. Hydrolysis efficiency was evaluated in each analytical run using hydrolysis controls that quantified within 7.2% of target. Analyte recovery from meconium was at least 77% with buffer extraction followed by solid phase extraction. The assay was linear from the method's limit of quantification of 20 ng/g to 2000 ng/g for buprenorphine and norbuprenorphine. Accuracy was >86% and precision >84% with no interference from 69 tested licit and illicit drugs and metabolites.

Total buprenorphine concentrations ranged from 24-297 ng/g with a mean (±SE) of 131 ± 27 ng/g and a median concentration of 110 ng/g. Free buprenorphine ranged from 24-240 ng/g (mean = 93 ± 23; median = 60 ng/g). One specimen, which contained 24 ng/g total buprenorphine, had free buprenorphine concentrations below the method's limit of quantification. The percent free buprenorphine was 35-82%, with an average of 64 ± 6%, indicating inter-subject variation in glucuronide conjugation. Matched-pair t-test of total and free analysis indicated a statistically significant higher concentration of total buprenorphine than free (mean difference = 49 ± 10 ng/g, n=9 pairs, t=4.788, 8df, p=0.001). Specimens contained higher concentrations of total and free norbuprenorphine, 324-1880 (mean = 754 ± 136 ng/g; median = 660 ng/g) and 331-1229 ng/g (mean = 610 ± 88 ng/g; median = 501 ng/g), respectively. Four specimens had >99% free norbuprenorphine. Three of these actually had lower total than free drug concentrations, but results were within ± 20%, the imprecision of the analysis. Another possibility could be the difficulty in completely homogenizing meconium. The remaining specimens (N=6) ranged from 53-89% free norbuprenorphine (mean 71 ± 6%). There was no statistically significant difference between the concentration of total norbuprenorphine and the concentration of free drug (mean difference = 143 ± 78 ng/g, t=1.840, 9df, p=0.099).

The free buprenorphine to free norbuprenorphine ratio was 0.14 ± 0.02 ng/g (range: 0.07-0.20 ng/g; median = 0.14) and the total buprenorphine to total norbuprenorphine ratio was 0.18 ± 0.03 ng/g (range: 0.05-0.33 ng/g; median = 0.16.). There is no statistically significant difference between these two ratios (p=0.37).

These findings impact the forensic community by providing the first data on buprenorphine and norbuprenorphine excretion in meconium following controlled drug administration to a cohort of pregnant women. These data provide valuable information on buprenorphine and norbuprenorphine concentrations and the extent to which these analytes may be conjugated in meconium. Additionally, these data allow for correlations to be examined between maternal dose and meconium concentrations and between meconium concentrations and neonatal outcomes, providing critical information for clinicians.

Buprenorphine, Meconium, Pregnancy

K51 Disposition of Nicotine and Metabolites in Human Meconium Following In Utero Tobacco Exposure

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After attending this presentation, attendees will learn the disposition of nicotine, cotinine, 3'-trans-hydroxycotinine, nornicotine and norcotinine in human meconium after in utero tobacco exposure, including relative analyte concentrations, metabolite ratios, and degree of glucuronidation.

This presentation will impact the forensic community, as this is the first quantification of nicotine and metabolites in meconium, the first data on the percentages of total and free nicotine and metabolites in meconium and the first report of the importance of nicotine as a biomarker of in utero tobacco exposure in meconium. These data will aid in the identification of prenatally tobacco exposed infants.

Approximately one-quarter of pregnant women smoke tobacco despite nicotine's known effects on fetal growth, lung and nervous system development, and increased risk of nicotine dependence in adulthood. Detection of cotinine in meconium by immunoassay is the primary means of monitoring *in utero* nicotine exposure. Recently, the first chromatographic assay for nicotine and metabolites in human meconium was developed and validated in our laboratory. Liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry in positive ion mode was employed to simultaneously quantify nicotine and metabolites, cotinine, OH-cotinine, norcotinine and nornicotine in meconium from 125 neonates.

Meconium (0.5 g) was homogenized with 3 mL of acidified methanol. After sonication, centrifugation, reconstitution in buffer and *E. coli* β-glucuronidase hydrolysis for 18 h at 37°C, solid phase extraction using a mixed-mode cation exchange column was performed. Limits of quantification (LOQ) were 1.25 ng/g for OH-cotinine, cotinine, and norcotinine and 5 ng/g for nicotine and nornicotine. Specimens were analyzed with and without β-glucuronidase enzymatic hydrolysis to determine total and free concentrations.

Fifty-nine specimens (47.0%) were positive for at least one free drug, with nicotine being the primary analyte detected (43.2%), followed by OH-cotinine (37.6%), cotinine (34.4%), nornicotine (12.0%) and norcotinine (9.6%). The highest percentage of specimens (20.0%) contained nicotine, cotinine and OH-cotinine, 8.8% were positive for all five analytes, and 8.8%, 0.8% and 2.4% for nicotine, cotinine and OH-cotinine only, respectively. No specimen was positive for only nornicotine or norcotinine. Average free drug concentrations of positive specimens (±SD) were 72.6 (±92.2) ng/g OH-cotinine, 69.9 (±70.3) ng/g cotinine, 59.6 (±76.3) ng/g nicotine, 4.5 (±3.7) ng/g norcotinine, and 11.8 (±6.2) ng/g nornicotine. Average total drug concentrations of positive specimens (±SD) were 99.2 (±118.2) ng/g OH-cotinine, 80.3 (±78.5) ng/g cotinine, 60.0 (±73.3) ng/g nicotine, 4.2 (±3.9) ng/g norcotinine, and 11.8 (±5.1) ng/g nornicotine. Two specimens had OH-cotinine concentrations greater than the 500 ng/g upper limit of quantification, but could not be reanalyzed due to lack of additional specimen. Statistically significant differences were shown between total and free OH-cotinine and cotinine using a paired t-test (P<0.05). Amongst positive specimens, the average percentage (±SD, range) of total OH-cotinine, cotinine, and nicotine present as glucuronide conjugates were 29.4 (±21.1, -19.4-73.3), 15.7 (±16.2, -18.6-54.5), and 4.2 (±12.4, -36.2-30.6), respectively. Free drug concentrations greater than total drug concentrations can be attributed to analytical imprecision and lack of homogeneity in the

matrix despite extensive mixing prior to sampling. OH-cotinine total drug and glucuronidation results should be interpreted with caution, as hydrolysis efficiency of authenticated OH-cotinine-O-glucuronide was determined to be 15% during method validation. Possibly, the high degree of OH-cotinine glucuronidation observed could be due to the presence of di-glucuronide or N-glucuronide species in addition to the O-glucuronide control that was tested for hydrolysis efficiency. Hydrolysis efficiencies for nicotine- and cotinine-N-glucuronide were greater than 80%. Average free drug metabolite ratios (±SD) were: OH-cotinine/nicotine 1.75 (±1.74) [median (range), 1.37 (0.055-8.67)], cotinine/nicotine 1.44 (±1.28) [1.00 (0.31-5.90)], norcotinine/nicotine 0.058 (±0.080) [0.028 (0.009-0.288)], nornicotine/nicotine 0.11 (±0.11) [0.08 (0.04-0.46)], OH-cotinine/cotinine 1.41 (±0.98) [1.18 (0.15-3.94)], and norcotinine/cotinine 0.08 (±0.03) [0.08 (.04-0.13)].

Meconium, Nicotine, Cotinine, In Utero

K52 MDMA, HMMA, MDA, and HMA Plasma Pharmacokinetics in Humans Following Controlled MDMA Administration

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Attendees of this presentation will be informed about the plasma pharmacokinetics of 3,4-methylenedioxymethamphetamine (MDMA or ecstasy), 4-hydroxy-3-methoxymethamphetamine (HMMA), 3,4-methylenedioxyamphetamine (MDA), and 4-hydroxy-3-methoxyamphetamine (HMA).

This presentation will impact the forensic science community by presenting data to improve the interpretation of MDMA and metabolite plasma concentrations.

The pharmacokinetics of MDMA after controlled oral dosing will be presented. These results are part of a larger investigation of the effect of MDMA on human brain activity and cognitive performance and the relationship of effects to plasma MDMA and metabolite concentrations.

Seventeen young adults, ages 18-27, volunteered for this Institutional Review Board-approved study. Eight African-American males, six African-American females, two Caucasian males, and one Caucasian female provided written informed consent. Volunteers received three doses of MDMA, 0 (placebo), 1.0 (low) and 1.6 (high) mg/kg MDMA, in a double-blind, within-subject, randomized and balanced design. 150 mg was the upper limit of dosing for safety purposes. Dosing was separated by a minimum of one week. Participants resided on a closed research unit and plasma was collected for 47-167 h after MDMA administration. A fully validated 2D GC/MS method simultaneously quantified MDMA, HMMA, MDA, and HMA in human plasma. Calibration curves were MDA, 1-100 ng/mL; HMA, 2.5-100 ng/mL; and MDMA and HMMA, 2.5-400 ng/mL. The lowest calibrator concentration was equal to the limit of quantification. WinNonlin was used to determine pharmacokinetic parameters. Paired t- and Wilcoxon Signed Rank tests were performed using SPSS v 14.0. $p < 0.05$ (two-tailed) was considered significant. Data are presented as mean ± standard deviation (SD).

In general, participants were positive for MDMA and HMMA by 30 min post-dose; MDA was quantifiable in all subjects by 1.25 h. HMA had a variable first detection time, ranging from 1.25-9 h. Mean maximum plasma concentrations (C_{max}) of 162.9±39.8 and 171.9±79.5 ng/mL were observed for MDMA and HMMA, respectively, after the low dose. After the high dose, mean MDMA C_{max} increased to 291.8±76.5 ng/mL, while mean HMMA C_{max} was relatively unchanged at 173.5±66.3 ng/mL. High inter-subject variability in C_{max} was observed. The highest individual C_{max} were 465.3 (MDMA) and 318.1 (HMMA) ng/mL. Mean MDA C_{max} were 8.4±2.1 (low) and 13.8±3.8 (high) ng/mL. HMA C_{max} were lower at 3.5±0.4 and 3.9±0.9 ng/mL after the low and high dose, respectively. C_{max} of all analytes except HMMA were significantly higher after the high dose. A comparison of MDMA and HMMA C_{max} revealed a significant difference after the high dose only (n=17, p=0.001), indicating non-linear HMMA pharmacokinetics. Mean time to maximum concentrations (T_{max}) after the low dose were MDMA, 2.4±0.6 h; HMMA, 1.8±0.7 h; MDA, 7.5±1.7 h; and HMA, 10.6±2.6 h. T_{max} did not significantly differ between dose for any analyte. 100% of participants were positive for MDMA, HMMA and MDA at 23 h after both doses. Similar patterns of detection were noted for MDMA and MDA; 48 h after the low dose, <25% of subjects were positive, while after the high dose, positivity increased to >80%. >90% were HMMA positive 48 h after the low and high doses; HMMA had the longest window of detection of up to 95 h, while HMA was not measurable beyond 47 h. Mean half lives ($t_{1/2}$) of MDMA were 6.9±3.4 h (range: 4.1-18.3) and 8.1±2.1 h (range: 4.7-13.3) after the low and high dose, respectively. HMMA mean $t_{1/2}$ was 11.5±5.5 h after the low and 13.5±2.7 h after the high dose. MDA $t_{1/2}$ were shorter than previous reports, at 10.6±4.3 (low) and 12.3±3.7 (high) h. HMA $t_{1/2}$ showed high variability due to low concentrations. Half-lives of all analytes except HMA were significantly longer after the high dose. Mean MDMA volume of distribution was 5.5 L/kg after both doses; clearance was significantly higher after the low (0.62±0.19 L/h/kg), as compared to the high (0.48±0.11 L/h/kg) dose (n=17, p=0.004).

Extended plasma collection, large sample size, and multiple doses permitted a comprehensive evaluation of MDMA, HMMA, MDA, and HMA pharmacokinetics. These data will impact the forensic science community by improving the interpretation of MDMA and metabolite plasma concentrations.

MDMA, Ecstasy, Pharmacokinetics

K53 Comparison of Various Liquid Chromatography–Mass Spectrometry Technologies for the Analysis of Forensic Toxicology Samples for Commonly Encountered Drugs

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After attending this presentation participants will have a greater understanding of how a variety of mass spectral technologies may be applicable to particular analytical challenges.

This presentation will impact the forensic science community conducting analysis of “drugs” by providing comparative data from a variety of mass spectral techniques on the same samples.

Introduction: A wide array of mass spectral and hyphenated mass spectral technologies is currently available for analysis of small molecules. It has become increasingly confusing for investigators to understand which technologies may be best for particular analytical applications. Specifically, this presentation will provide an overview of LC/QqQ (triple quad), LC/QTOF (time of flight), LC/TOF, LC/MS (single quad), LC/MS ion trap and DART-TOF (Direct Analysis in Real Time, Time of Flight MS) technologies. These technologies will be applied to the same samples to allow for the comparison of what types of technologies are advantageous for which types of applications. Samples will include postmortem specimens and DUID specimens.

Method: Typical forensic samples, blood from death and DUID cases, were prepared in sufficient amounts such that the same extracts could be

analyzed on the various fragmentation and detection instruments. Samples were extracted in a manner appropriate for the matrix and suitable for the various ionization methods. For DART analysis, blood samples were introduced without sample preparation and with the same sample preparation as for LC/MS. Samples were run on an AccuTOF-DART system located at RTI International and on LC/MS systems located at the University of Miami forensic toxicology laboratory and at various Agilent application laboratories.

Results: Representative chromatograms and mass spectra will be presented. The various strengths and limitations of quadrupole-based technologies for quantification and SRM/MRM identification will be compared with identification of compounds using accurate mass and MSn techniques. The presentation will also discuss how individual technologies can accommodate both identification and quantification. Specifically, the different mass spectrometers will be discussed in light of each instrument's strengths and weaknesses for providing forensically-acceptable, highly sensitive screening and identification results.

Conclusions: These comparative MS/MS data provide examples appropriate to the challenges faced in forensic toxicology and demonstrate what various instruments are able to achieve. This presentation will provide examples from a variety of mass spectral technologies from at least two manufacturers and will hopefully provide the groundwork for further comparative studies to include upcoming technologies and more manufacturers.

Analytical Toxicology, Postmortem Toxicology, LC/MS

K54 New Strategies to Apply LC/MS/MS for the Quantitation and Confirmation of Hundreds of Substances Relevant in Forensic Toxicology

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The goal of this presentation is to present the comparison of different LC/MS/MS technologies used for screening and followed by library searching to detect drugs of abuse and pharmaceuticals.

This presentation will impact the forensic science community by demonstrating how LC/MS/MS will replace LC/UV screening methods.

Introduction: With about 5% of the population between the ages of 15 and 64 (~200 million people) using illicit drugs, and thousands of drug intoxications per year in the western world alone, fast screening methods for drugs and pharmaceuticals are necessary for the detection of xenobiotics in forensic intoxication cases. Screening methods usually include immunoassay tests, available only for a small number of substance classes, Gas Chromatography (GC) especially with Mass Spectrometric (MS) detection, or Liquid Chromatography (LC) with Ultraviolet (UV) detection. While GC requires typically extensive clean-up steps with derivatization, LC is ideally suited for polar compounds but UV detection lacks the necessary specificity and methods require long run times to minimize the potential for co-elution. Since 1999, screening for drugs with LC/MS and LC/MS/MS has made progress with mass spectral library searching to confirm detected drugs.

Experimental and Results: This presentation compares LC/MS(/MS) screening strategies using different mass spectrometric detection techniques such as Time of Flight (TOF), Single and Triple Quadrupole, Ion Trap and Hybrid Triple Quadrupole Linear Ion Trap. These MS technologies are compared regarding their ability to screen for a large number of compounds, sensitivity, selectivity, and the possibility of using mass spectral libraries to confirm the presence of detected analytes. Additionally the possibility of transferring once generated libraries to other instruments is discussed. A mass spectral library with more than 1200 substances was generated by injection of standard solutions using standardized Collision Energies (CE) of 20, 35, and 50. In addition a CE of 35V with a Collision Energy Spread (CES) of 15V was used. Data presented were acquired on different mass spectrometers including API 3200™, 3200 Q TRAP® and QSTAR® LC/MS/MS systems in different quadrupole, TOF, and ion trap scan modes. Electrospray Ionization (ESI) was used to ionize all investigated compounds including drugs of abuse, pharmaceuticals, and metabolites. A Shimadzu Prominence HPLC with reversed phase column was used with a standard eluent of water and acetonitrile with a buffer of formic acid and ammonium formate.

Comparative analysis of 300 compounds and extracts of urine and blood sample were used to investigate different MS technologies and their advantages and disadvantages when used for the screening in forensic toxicology.

Conclusion: It was found that the combination of highly selective and sensitive Multiple Reaction Monitoring and fast and sensitive Enhanced Product Ion Scan on a Hybrid Triple Quadrupole Linear Ion Trap is the most powerful LC/MS/MS technique to screen for a large number of unknown compounds and confirm their presence by library searching in forensic samples.

LC/MS/MS, Screening, Library Searching

K55 Whole Blood/Plasma Cannabinoid Ratios in Daily Cannabis Users After Multiple Years of Frozen Storage

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After this presentation, attendees will be informed of whole blood/plasma cannabinoid ratios in authentic specimens (N = 187 pairs) stored frozen for multiple years, and will learn of the first data on intra-subject whole blood/plasma Δ9-tetrahydrocannabinol (THC) and 11-nor-9-carboxy-THC (THCCOOH) ratio variability.

This presentation will impact the forensic science community by providing the first reference of cannabinoid whole blood/plasma ratios from aged authentic specimens and the first data on intra-subject whole blood/plasma THC and THCCOOH ratio variability.

After this presentation, attendees will be informed of whole blood/plasma cannabinoid ratios in authentic specimens (*N* = 187 pairs) stored frozen for multiple years, and will learn of the first data on intra-subject whole blood/plasma Δ9-tetrahydrocannabinol (THC) and 11-nor-9-carboxy-THC (THCCOOH) ratio variability. THC, 11-hydroxy-THC (11-OH-THC) and THCCOOH whole blood/plasma ratios are approximately 0.5, due to high affinity for albumin and/or lipoproteins. Few studies report whole blood/plasma cannabinoid ratios determined from direct comparison between simultaneously collected authentic specimens. In only one report were ratios determined from aged specimens and none report intra-subject ratio variability. Here, whole blood/plasma cannabinoid ratios in authentic specimens collected during a clinical study and stored in polypropylene at -20ºC for 2.9 to 5.6 years are investigated. Also reported are the intra-subject THC and THCCOOH whole blood/plasma ratio variability in 13 participants by direct comparison of specimens collected simultaneously during one week of cannabis excretion.

Thirty-two daily cannabis users (aged 19 - 38, 50% male, 84% African American) provided written informed consent for this IRB-approved study, and resided on a closed clinical research unit for seven days of monitored drug abstinence. Whole blood and plasma were collected simultaneously upon unit admission and every 24 h thereafter. Specimens were collected into Vacutainer tubes containing anticoagulant and transferred to polypropylene cryotubes for long term (-20ºC) storage. Cannabinoids were extracted

by SPE (Clean Screen® ZSTHC020 extraction columns, United Chemical Technologies) and derivatized with BSTFA + 1% TMCS. Extracts were injected on an Agilent 6890 GC/5973MSD (operated in EI/SIM mode) retrofitted with a Dean's switch and cryotrap. Two calibration curves (low, 0.125 – 25 and high, 25 – 100 ng/mL) were constructed with $r^2 > 0.99$. Plasma limits of quantification (LOQ) were 0.25 ng/mL for THC and THC-COOH and 0.5 ng/mL for 11-OH-THC. Whole blood LOQ were 0.25 ng/mL for all analytes.

Overall mean ± SD whole blood/plasma ratios were 0.39 ± 0.17 ($N = 75$, median 0.39, range 0.08 – 0.77), 0.55 ± 0.22 ($N = 17$, median 0.56, range 0.22 – 0.90) and 0.45 ± 0.29 ($N = 187$, median 0.37, range 0.11 – 1.53) for THC, 11-OH-THC, and THCCOOH respectively. Mean whole blood/plasma ratios for THC and THCCOOH were determined for 13 subjects that had a least three paired specimens with THC and THCCOOH greater than LOQ (Table 1). A paired samples t test ($\alpha = 0.05$) including only the 13 participants' paired THC and THCCOOH ratios revealed that the mean whole blood/plasma THC ratio was significantly lower than the correspon-ding mean THCCOOH ratio ($p < 0.01$). Four of 13 participants mean THCCOOH ratios ($N = 7$ each) were > 0.8. Removing these potential outliers yielded a non-significant difference ($p = 0.087$) between THC and THCCOOH mean ratios of 0.36 ± 0.10 and 0.46 ±, 0.13, respectively.

Table 1: Average of intra-subject mean ($N = 3 – 7$) whole blood/plasma THC and THCCOOH ratios from 13 participants over one week.

	N	Mean ± SD	Median	Range
THC	13	0.40 ± 0.11	0.40	0.13 – 0.56
THCCOOH	13	0.63 ± 0.28	0.56	0.28 – 1.15

These data impact the forensic community by providing the first reference of cannabinoid whole blood/plasma ratios from aged authentic specimens and the first data on intra-subject whole blood/plasma THC and THCCOOH ratio variability. The overall mean whole blood/plasma THC ratio was lower than previously reported, which may be explained by cannabinoid binding to whole blood proteins and/or container surfaces during storage. Mean intra-subject whole blood/plasma THC ratio was significantly lower than the corresponding mean THCCOOH ratio; however, further research may be necessary because data may be skewed by mean ratios >0.8 in four participants.

Cannabinoids, Ratio, Whole Blood

K56 A One-Year Study of Cocaine and Heroin in Waste Water Plants in Florence, Italy

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The goal of this presentation is to present data on a one-year study of cocaine and herion concentrations in waste water plants in Florence, Italy.

This study will impact the forensic science community by proving once again the potential of the analysis of drugs and metabolites in waste water as a tool in monitoring drug abuse and, especially, in the identification of trends in consumption habits.

Determination of drugs and metabolites in wastewater collecting plants (WWP) is a newly and efficiently developed strategy in the assessment of substance use and abuse in several different countries. In particular, in the last years, determination of cocaine and other drugs in wastewater was proposed to estimate *per capita* consumption with better reliability than traditional markers (as epidemiological studies, drug confiscations, crime statistics). Although an inter-laboratory validation of methods of analysis and data elaboration (especially in relating water concentration with population) is needed before this can be used as a widespread monitoring tool, the analysis of wastewater from a specific collection site by a validated method over a certain period of time can be helpful in the identification of trends in drugs use.

The amount of cocaine was determined (measured as the sum of cocaine and benzoylecgonine) and heroin (measured as morphine) over one year (July 2006-June2007) in wastewater of the City of Florence. Analytical results were used to evaluate a trend in the use of these drugs and to speculate on the impact of tourist flow on cocaine/heroin consumption in the city of Florence.

Wastewater was collected every month at two different WWPs located on opposite sides of the Arno River before any treatment. Three liters of water were analyzed by solid phase extraction (on Bond Elute LRC Certify, Varian Inc. Lake Forest, CA, according to the manufacturer's instructions for basic drugs with minor modifications), followed by N-Methyl-N-trimethylsilyltrifluoroacetamide derivatization and gas chromatography-mass spectrometry (GC-MS). Cocaine (COC), benzoylecgonine (BE) and mor-phine (MOR) were identified in selected ion monitoring mode (ions 82, 182, 272, 303 for COC, 82, 240, 346, 361 for BE, and 324, 401, 414, 429 for MOR). Four-point calibration curves were prepared (from 25 to 200 ng/L for COC and BE and from 22 to 212 ng/L for MOR) and accuracy and precision were calculated by repeatedly injecting three quality control (QC) points (25, 50, 150 ng/L for COC and BE, 22, 37, 153 for MOR). The analytical method was found to be linear for all substances in the range of interest (COC: slope: 44.72 ± 2.20, intercept: 2.39 ± 5.63, R^2: 0.9920 ± 0.008; BE: slope: 320.96 ± 39.36, intercept: 17.55, R^2: 0.9853 ± 0.016; MOR: slope: 515.54 ± 28.93, intercept: 3.8 ±7.49, R^2: 0.9975 ± 0.002). For the three substances, accuracy and precision results were better than 18.4% bias and 14.2% relative standard deviation at the lower QC and better than 10.2% bias and 14.8% relative standard deviation at low and high QC.

Cocaine (calculated as COC + BE equivalents) was assessed to be used in the city of Florence in the range between 42.84 and 82.54 g/day (mean: 59.05 g/day, median: 55.55 g/day), with the highest amounts in August (82.54 g/day), December (78.24 g/day), and March (78.79 g/day). The lowest quantities were retrieved in September (42.84 g/day), October (43.70 g/day), and January (44.69 g/day). Heroin (calculated as morphine equivalents) use was estimated to be used between 2.92 and 17.17 g/day (mean: 9.80, median: 10.56 g/day). The highest amounts were observed in January, March, and April (14.78, 15.58, and 17.17 g/day, respectively) and the lowest in July, September, and October (2.92, 3.93, and 3.84 g/day, respectively). The analysis of cocaine and heroin in surface water over a 12-month period is, to our knowledge, unprecedented and, on the basis of these preliminary data, it is possible to: (i) recognize an increment in the use of heroin in the period taken into consideration (with an average of 4.7 g/day in the first trimester and 12.8 in the last one), (ii) speculate that cocaine use seems higher when the tourist flow is more intense (August and December, in particular), and (iii) assume that heroin does not appear to be influenced in this way (highest amount from January to April).

This study proves once again the potential of the analysis of drugs and metabolites in wastewater as a tool in monitoring drug abuse and, especially, in the identification of trends in consumption habits. Finally, the project is still ongoing and is being extended toward more substances and metabolites.

Waste Water Plants, Cocaine, Florence

K57 Analysis of Antipsychotic and Antidepressants in Whole Blood by LC/MS/MS

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After attending this presentation, attendees should have a more thorough understanding of the capabilities of screening for antipsychotic and antidepressants in whole blood by LC/MS/MS.

This presentation will impact the forensic science community by providing analytical information to members of the medical examiner toxicologist community and to individuals that may take an interest in the analysis of these types of compounds to determine their concentrations in whole blood.

The objective of this research was to develop an LC/MS/MS method for screening and confirmation of various antidepressant and antipsychotic drugs in whole blood. These types of compounds are consistently among the most commonly prescribed medications and as such can be routinely encountered in the performance of therapeutic drug monitoring or medical examiner toxicological examinations. The analysis of these types of drugs may be hampered by laborious extraction procedures, extended analytical analysis times, and even the need for multiple analyses. A generalized sample preparation and analysis method by HPLC tandem MS is presented.

This method describes the simultaneous analysis of more than ten antipsychotic, antidepressant, and structurally similar medications. Whole blood is subjected to protein precipitation. The corresponding supernatant was subsequently analyzed using reversed-phase HPLC with MS/MS detection. The column used was an Allure PFP Propyl from Restek Corporation. The mobile phase consisted of a binary mixture for a gradient. Mobile phase A was aqueous 1.0 mM ammonium acetate with 0.05% acetic acid. Mobile phase B was 95% Acetonitrile and 5% water with 1.0 mM ammonium acetate and 0.05% acetic acid. Detection was by single reaction monitoring for each compound. Confirmation was by multiple reaction monitoring of two transitions for each compound.

All of the analyzed antipsychotics, antidepressants and structural analogs were analyzed in a single method. The analytical run time was complete within 15 minutes. Detection limits of the individual drugs and their observed linear ranges are presented. Linear ranges for the drugs generally covered at least two orders of magnitude. The limits of detections for many the compounds allow for application of the method to monitoring therapeutic concentrations.

The method provides an accurate and reliable means for detection and confirmation of various antipsychotic and antidepressant drugs in a whole blood matrix. The use of a precipitation as a means of sample preparation is less laborious and more time effective than classical liquid-liquid or solid phase extraction. In addition the versatility associated with HPLC and tandem mass spectrometry makes it likely that addition drugs could be added to expand the scope of the analysis with few modifications.

Antipsychotics, Whole Blood, LC/MS/MS

K58 Analysis of Postmortem Blood and Tissue by AccuTOF™ DART™

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After attending this presentation, the attendees will understand the potential strengths and weaknesses of the application of the AccuTOF™ DART™ system to screening of postmortem blood and tissue specimens.

The presentation will impact the forensic community by providing information about a novel technology applied to screening of postmortem blood and tissue samples.

Introduction: The innovative use of direct analysis real time (DART) coupled with time-of-flight (TOF) mass spectrometry has the potential to be of great value in the area of postmortem blood and tissue analysis. Generally, the technology applied by the AccuTOF-DART™system allows for the analysis of many samples without the need for sample preparation or solvents. Additional benefits of TOF-DART include: (1) use of limited sample size, (2) simultaneous screening and identification of a myriad of drug classes, and (3) minimal time for analysis. This system was used to analyze blood and tissue samples collected from various medical examiners' offices (Maricopa County Office of the Chief Medical Examiner, North Carolina Office of the Chief Medical Examiner, Washington State Toxicology Laboratory). The purpose of this study is to evaluate the AccuTOF-DART™system as a novel approach to expeditiously screen postmortem toxicology samples.

Methods: More than 23 blood and tissue specimen cases were analyzed both with and without minimal sample preparation and extraction. These samples were previously analyzed by traditional postmortem techniques and collection of samples contained 32 different drugs based on the previously reported results from traditional postmortem analysis. All tissue specimens were homogenized in deionized water (1:4). Initially, blood and liver samples were extracted in n-butyl chloride, evaporated, and reconstituted in butyl acetate. As an alternative extraction and sample preparation, the samples were extracted in acetonitrile, evaporated and reconstituted in acetonitrile. Samples were analyzed, for the corresponding M + H ion, by AccuTOF-DART™ mass spectrometry in positive mode. TOF-DART results were compared with the results reported from previous analysis of the cases by their respective toxicology laboratory.

Results: Initial attempts with directly introducing blood and tissue specimens resulted in no detection of corresponding M+H ions from drugs of interest. It was apparent that a sample extraction or minimally a precipitation of proteins was necessary for detection of compounds. With TOF-DART analysis of the representative samples, the results for the solvent-extracted blood and tissues and the protein-precipitated postmortem samples were comparable. In many instances, TOF-DART analysis of a sample produced expected M+H ions for a particular drug of interest, but not for another drug, previously detected using traditional postmortem analysis. For example, amitriptyline previously quantitated at 23 mg/kg in the liver homogenate, was detected by TOF-DART; however, metaprolol and nortriptyline (reported at 25 mg/kg and 35 mg/kg, respectively) were not detected. For example methadone, was consistently detected as low at

0.18 mg/L in aortic blood and 2.1 mg/kg in liver from the same case. In contrast, benzoylecgonine, like other drugs, was undetectable by TOF-DART when it was detected by traditional methods as low as at 6.9 mg/L. This unpredictability was found to occur in both blood and tissue samples.

Conclusions: Although the AccuTOF-DART™system has the ability to detect the presence of analytes by direct analysis, current indications show that drugs are not detected in postmortem samples without extraction or protein precipitation. Even with extraction and some concentration, many drug were not detected at high or low levels in both blood and tissue samples. It has been previously reported detection of many of the drugs, such as cocaine, at lower levels when drugs spiked into blank blood or urine, but these same drugs in the more complex matrix of postmortem blood and tissue were not as readily detectable. The samples analyzed in this study are archived postmortem samples and the stability of the drugs in these particular samples was not confirmed by traditional postmortem methods after TOF-DART. The AccuTOF-DART™system is a novel approach in the analysis of compounds; however, due to the nature of many postmortem specimens it does not have the sensitivity to detect the presence of many drugs.

AccuTOF™ DART™, Postmortem, Toxicology Screening

K59 Statistical Interpretation of Meprobamate Concentrations in Bone Marrow, Vitreous, and Bile

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Upon completion of this presentation, participants will have some tools to interpret postmortem meprobamate concentration in bone marrow, vitreous and bile. The proposed methodology could be applied to interpret the concentrations measured in other biologic matrices.

In numerous cases of toxic death investigations, the interpretation of blood concentrations is difficult (postmortem redistribution, putrefied bodies) or impossible (lack of blood sample). This presentation will impact the forensic community by enabling an interpretation of meprobamate concentrations measured in sample types other than blood sample.

The interpretation of concentrations in samples other than blood is complex due to the lack of reference ranges. The presented statistical methodology enables the decision of an intoxication or a therapeutic case with a quantified risk of error, which is very important when discussing the results in court.

The presented study is based on 116 forensic cases. On the basis of blood concentration, 70 cases were classified as therapeutic blood concentrations and 46 as toxic blood concentrations. For each case, at least one of the following sample types was collected during the autopsy: bile (n=107), right vitreous (n=40), left vitreous (n=43), and bone marrow (n=51). Meprobamate was quantified by GC/MS. For each sample type, the average concentration and the standard deviation showed that the meprobamate concentrations between the toxic and therapeutic populations were statistically significantly different.

Modeling of the toxic and therapeutic populations allowed the definition of a toxic threshold with less than 5% false positives. Multivariate analysis, such as Principal Components Analysis (PCA) and Partial Last Square Data Analysis (PLSDA) showed that it was possible to distinguish therapeutic cases and the toxic cases by simultaneous use of the concentrations measured in the 4 alternative matrices.

Practical applications of these results on some cases will be presented, as well as cases previously published in the international literature.

Meprobamate, Bone Marrow, Forensic Toxicology

K60 Rapid Determination of N_2O in Postmortem Biological Samples: A Case of Serial Fatal Poisoning

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After attending this presentation, attendees will be briefed on eight cases of fatal poisoning which occurred during general anesthesia.

This presentation will impact the forensic community and/or humanity by demonstrating the presence of N_2O in forensic biological samples by headspace- gas chromatography analysis using ECD detector (HS-GC/ECD).

Case History: In a public hospital during anesthesia, eight accidental deaths occurred due to an erroneous replacement of O_2 with N_2O. Four were females and four were males with a mean age of 77.75 years (range 67-85). Five of the decedents showed cardiovascular diseases, two had lung disease and one had gastrointestinal disease. During anesthesia, all were exposed to N_2O for a mean period of 58,25 min (range 25-125 min) until they expired.

Goal: It is known that Nitrous oxide (N_2O) is an asphyxiant at high concentrations [ACGIH 1991]. Determination of the cause of death in gaseous asphyxiation cases is very difficult due to the variation in circumstances during the event. To clarify the cause of death and identify the factors involved in asphyxia, gases from different lines were characterized and N_2O concentrations in postmortem biological samples (air and tissue samples), collected after 19 days postmortem (range 6 – 31) were analyzed.

Methods: Analyses, carried out on the gas samples both from the O_2 and the N_2O lines in the surgery room, confirmed the incorrect connection of the lines. In fact, gas samples from O_2 lines showed the presence of pure N_2O, while in those collected from the air lines there was pure O_2 with a low percentage of N_2O (less then 0.1%). Analysis of gas samples from the lines supplying each bed, produced the same results.

The analyses performed on the postmortem biological samples, showed an abnormal concentration of N_2O. Particularly, air samples collected from the stomach of all patients during autopsy revealed concentrations from 0.12 mM to 1.9 mM N_2O corresponding to 0.30 % and 4.55%, respectively. All samples were collected in duplicate and stored in 100 ml syringes until analysis. Calibration was carried out using air samples with a known amount of N_2O.

The presence of high levels of N_2O was found in urine, blood, kidney and liver. Results showed a variation in the distribution of the gas consistent with its solubility in the different tissues.

Results confirmed that the air supply lines were indeed switched. The data also indicate that N_2O could be detected in biological samples 31 days postmortem due to the high exposure concentrations.

Therefore, this report presents valuable findings for the correct diagnosis of the cause of death and helps to clarify the true nature of the cause of death.

Nitrous Oxide, Anesthesia, Accidental Death

K61 Deaths Attributed to Intravenous Use and Nasal Inhalation of Oxycodone

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After attending this presentation, attendees will understand the routes of oxycodone administration, understand issues in cases of atypical routes of drug administration, and understand some factors that could affect oxycodone toxicity.

This presentation will impact the forensic science community by providing forensic toxicologists and pathologists additional factors to consider in interpreting oxycodone drug levels following non-oral routes of administration.

Oxycodone is a semisynthetic narcotic analgesic derived by chemical modification from codeine. It produces potent euphoria, analgesic and sedative effects, and has a dependence liability similar to morphine. Two cases of death from oxycodone are presented: one by intravenous and one by nasal insufflation.

The first case was a 50-year-old Caucasian male who was pronounced dead in hospital. A full autopsy was performed < 24 h after death. Autopsy findings included extensive systemic foreign body granulomatosis consistent with IV drug use. The second case was a 28-year-old male found deceased at a friend's home. A friend at the scene reported that the decedent "snorted" the drug. A full autopsy was performed <24 hours after death. Autopsy findings included pulmonary oedema and moderate diffuse cerebral swelling. Blood and urine specimens were collected at autopsy for toxicological analysis.

Blood and urine specimens were subjected to a thorough qualitative analysis. Screening was performed for illicit drugs including opiates, cocaine, barbiturates, benzodiazepines, amphetamines, phenycyclidine, and cannabinoids by immunoassay. Acidic and neutral drugs were screened for by liquid-liquid extraction followed by GC-MS electron impact detection. Volatile alcohols were assayed by GC-FID. Qualitative analysis in urine identified oxycodone and cannabinoids in both cases. Quantitation of oxycodone and 11-Nor-Delta^{9}Tetrahydrocannabinol-9-CarboxylicAcid (THC-COOH) in urine and oxycodone in blood were performed by GC-MS. Oxycodone and its deuterated internal standard were extracted at pH 6.0 using solid phase extraction techniques. The eluant is evaporated, and the resulting residue is dissolved in pyridine. Acetyl derivatives of oxycodone are then formed by adding acetic anhydride and heating the mixture for 30 minutes at 50°C, then dried under nitrogen. The resulting residue is reconstituted in ethyl acetate and subsequently analyzed by gas chromatography/mass spectrometry using single ion monitoring; Oxycodone - 357, 314, 358 m/z; and Oxycodone-d_3 - 360, 317, 361 m/z.

The concentrations of oxycodone found in blood and urine for cases #1 were 0.518 mg/L and 21.7 mg/L respectively. The THC-COOH was 0.020 mg/L in urine. The concentrations of oxycodone found in blood and urine for cases #2 were 0.050 mg/L and 6.58 mg/L respectively. The THC-COOH was 0.081 mg/L in urine.

The usual adult oral dose is 2.5-5 mg every 6 hours, although patients with moderately severe pain may take 10-30 mg every 4 hours. Published pharmacokinetic studies involving oxycodone show that plasma concentrations are generally less than 0.100 mg/L. For example, the peak plasma concentrations in 12 patients receiving a 10 mg oral dose averaged 0.030 mg/L. There is little reported on the lethal levels of oxycodone in blood when administered intravenously or by nasal inhalation. For oral oxycodone alone, a minimum lethal level of 5.0 mg/L has been suggested, and fatal concentrations involving oxycodone and at least one other depressant drug have been reported at 0.60 mg/L. Although the concentration of oxycodone in these cases was lower, it is well known that for other opiates the minimum lethal level can be considerably lower when administered intravenously or by insufflation than when orally administered.

Based on autopsy findings, investigation at the scene, patient history, and toxicology findings, the cause of death in case #1 was ascribed to oxycodone administered by intravenous route; case #2 was ascribed to oxycodone administered by nasal inhalation; and the manner of death in both cases was determined to be accidental.

Oxycodone, Intravenous, Nasal Inhalation

K62 The Controversy of Death Involving Drugs of Abuse and TASERs®

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The goal of this presentation is to present the case history and toxicological findings of a fatality involving the use of a TASER® stun gun on an individual confirmed to have used cocaine. After attending this presentation, attendees will gain insight into the use of incapacitating devices by law enforcement personnel, the detection of illicit drugs in body fluids, and the contribution of each in rendering an opinion in a death investigation.

This presentation will impact the forensic community and/or humanity by reviewing a multi-jurisdictional death investigation involving an electrical weapon with the concurrent use of a drug of abuse. This combination continues to be prevalent in North America and controversial in regards to cause and manner of death.

The authors present the case history and toxicological findings of a fatality involving the use of a TASER® stun gun on an individual confirmed to have used cocaine. After attending this presentation, attendees will gain insight into the use of incapacitating devices by law enforcement personnel, the detection of illicit drugs in body fluids, and the contribution of each in rendering an opinion in a death investigation.

Numerous accounts of law enforcement personnel using TASER® stun guns to subdue suspects in the field and in custody have been reported. The prevalence of abstracts presented at recent AAFS meetings regarding the use of TASERs® attests to the continuing debate as to whether they are lethal weapons, non-lethal weapons, or something in-between.

A 36-year-old male was confronted by officers from a local township police department responding to reports of a suspicious person in the area pounding on doors and windows. The combative male resisted arrest and was subdued with at least five "drive stuns" (without probes) in the small of the back. (Note: one of the officers was shocked twice by his own TASER® during the confrontation). The male subject was also shocked in the abdominal region by another officer with the TASER® probes attached. The male subsequently fell into an Oriental pond containing approximately 2.5 feet of water. The officers promptly removed him from the water. He became unresponsive and was immediately transported to the nearest medical center where he was pronounced dead approximately 30 minutes later. An autopsy was performed and specimens were collected for toxicology testing (blood, bile, gastric and vitreous humor).

Routine toxicological analyses of postmortem blood and vitreous humor were conducted to aid in the determination of cause and manner of death. Thorough examination of the decedent was performed so as to ascertain whether the death was attributable to the use of TASER® stun guns or to the presence of illicit and/or prescription drugs or some other cause or combination of causes. The analytical procedures employed included immunoassays, spot tests, and gas chromatographic methods utilizing flame ionization, nitrogen-phosphorus, and mass spectrometric detection. The presence of cocaine/metabolites was indicated in the vitreous humor via im-

munoassay. Identification and quantitation of cocaine/metabolites was performed in the femoral blood revealing the following concentrations: 1465 ng/ml cocaine, 3036 ng/ml benzoylecgonine, positive ecgonine methyl ester. The presence of alcohol or other drugs of significance were not detected. The cause of death in this case was determined to be excited delirium due to cocaine intoxication and the manner of death accidental.

Use of incapacitating devices was temporarily suspended by the police department pending an investigation by the county sheriff's office. Each of the police department's 16 TASERs® was examined to ensure proper operation. Reporting of this incident prompted a number of law enforcement agencies in the region to conduct reviews of their respective TASER® policies. According to the manufacturer, TASER International, TASERs® are deployed as a non-lethal alternative to deadly force. However, other groups such as Amnesty International and the American Civil Liberties Union believe that TASERs® pose a serious health risk and should be considered as a contributing factor in TASER® related deaths.

TASER®, Cocaine, Death

K63 Morbidity Involving the Hallucinogenic Designer Amine 2C-I: Case Report

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After attending this presentation, attendees will be familiar with a new class of hallucinogenic synthetic amines and a severe complication that occurred in one case involving 2C-I.

This presentation will impact the forensic community and/or humanity by familiarizing attendees with a new class of hallucinogenic drugs and one possible adverse effect of these drugs. It will also describe a successful method for detecting these designer amines.

The 2C* family of designer amines are derivatives of the natural compound b-phenethylamine. They contain methoxy groups in positions 2 and 5 and a hydrophobic 4-substituent (iodine in 2C-I, bromine in 2C-B, etc). The name "2C" comes from the two carbon atoms that separate the amine from the phenyl ring. The 2C* drugs have hallucinogenic properties and are sometimes incorrectly sold as MDMA. Little is known about the pharmacological and toxicological properties of the 2C* drugs, but it is known that they show affinity to type-2 serotonin (5-HT_2) receptors, acting as agonists or antagonists similarly to other hallucinogenic drugs.

In an adverse event involving one of the 2C* drugs, a 39-year-old woman presented to the emergency department on New Years day after a night of partying with diminishing mental status, agitation, hypothermia, hypertension, vasoconstriction, and hemorrhagic stroke. She was unresponsive and had extensor posturing. Her friends provided a history of MDMA (ecstacy) and 2C-I ingestion, the latter of which the patient reportedly synthesized at home using a recipe from the internet. A high performance liquid chromatography (HPLC) rapid UV scanning method (BioRad REMEDi) could not detect MDMA or MDA due to an interfering substance. However, a method using liquid chromatography tandem mass spectrometry (LC-MS/MS; Applied Biosystems 3200 QTrap) with selected reaction monitoring followed by a linear ion trap full scan was able to detect and identify both MDA and 2C-I in the patient's urine. MDA is a minor metabolite of MDMA and also an independently used drug. The absence of MDMA suggests that the patient ingested MDA alone and not MDMA, as was stated in the history. A head CT scan revealed that the patient had a congenital cerebrovascular abnormality (Moyamoya) that put her at a higher risk for stroke. Hypertension and stroke following MDMA ingestion has been well described. Similar reports are not available for MDA and 2C-I, perhaps because such exposures are less common and/or less often identified. The patient had an extended stay in the ICU, and six months later could follow commands but not speak. Despite these modest improvements in mental status, the patient remains severely disabled and requires total care.

In conclusion, associating clinical syndromes with use of illicit drugs by relying solely on self reporting or that of family members or involved bystanders may not be reliable, nor is laboratory analysis that does not address a broad spectrum of designer amines. This is potentially the first adverse event reported for 2C-I, though the possibility of 2C-I being coincident to MDA toxicity will also be discussed.

2C-I, MDA, LCMS

K64 Fentanyl in Blood and Head Hair From Postmortem Cases

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The goal of this presentation is to assess the prevalence of fentanyl in blood and corresponding head hair specimens in postmortem cases.

Routine testing methodologies in hair and blood analysis may not include fentanyl. With the use of transdermal delivery systems and potential lacing of street drugs with fentanyl, a need to develop a rapid and sensitive assay for this drug became apparent.

Specimens: Biological Specimens were obtained during the medicolegal death investigation process at The Office of the Cuyahoga County Coroner, Cleveland. Cases were included if there was a history of heroin and/or (transdermal) fentanyl use or fentanyl was detected in any routine assay, such as the basic drug screen.

***Methods* - Blood:** Biological Fluid testing was performed at The Office of the Cuyahoga County Coroner, Cleveland. Blood (heart or femoral) were assayed by solid phase extraction followed by gas chromatography/mass spectrometric analysis in the selected ion monitoring mode. Norfentanyl, fentanyl, alfentanil and sufentanil were included in the assay. Matrix matched calibrators were assayed at 1, 2, 5, 15, and 25 ng/mL with deuterated (d_5) fentanyl and norfentanyl as internal standards. A negative and positive control at 10 ng/mL fentanyl were assayed with each batch. The coefficient of determinations (r^2) were typically >0.99. The linear range of the assay was 1-50 ng/mL. Within (n=13) and between (n=5) day precision for fentanyl at 15 ng/mL was 4.28%CV and 6.52% CV, respectively. Accuracy at 5 ng/mL was 93.2% (n=5).

HAIR: Hair testing was performed at Immunalysis Corporation, Pomona, CA. A screening procedure for the detection of several medications using ELISA included washing 10 mg specimens briefly with acetone and air drying. Following cutting, 0.025M phosphate buffer (1.5 mL) was added. The samples were sonicated at 75°C for three hours, 0.2 mL of supernatant was removed and 0.8 mL of bovine serum albumin (BSA) added to dilute the sample 1:5. A specific aliquot of the extract was used for the ELISA analysis depending on the drug. Presumptively positive samples were re-aliquoted, washed, cut and sonicated in 0.025M-phosphate buffer (pH 2.7; 1.5 mL) for two hours at 75°C with corresponding internal standard. The buffer was removed, and 0.1M sodium phosphate buffer (pH 6.0; 1 mL) added; the samples were subjected to solid-phase extraction. Confirmation was achieved using two techniques. For 2-dimensional GC/MS, the extracts were reconstituted in ethyl acetate (40 µL) and transferred into auto sampler vials. The ions monitored were 250.2 and 151.1 for deuterated (d5) fentanyl; 245.2, 146.1 and 189.1 for fentanyl with a dwell time of 70 ms. The system was operated in electron impact mode. Alternatively, for LC/MS/MS analysis, the instrument was operated in atmospheric pressure chemical ionization positive mode and the collision gas was nitrogen. Two transitions were monitored (337.4 to 188.3; 337.4 to 105.3) and a ratio calculated so as to increase confidence in the result. Both procedures had a limit of quantitation of 10 pg/mg.

Results: 23 cases were identified for inclusion in the study. The majority of decedents were white (83%) and the sexes were evenly divided (52% male). The age range was 30-94 years with a mean±SD of 58.7±18.0, and a median of 51 years. Twelve individuals had a history of transdermal patch use, 9, a history of heroin use, 1 individual both and one with no history. Fentanyl was detected in the blood of 14 cases (n=21) in a concentration range of 1-33 ng/mL (8.78±9.25, 5.50 ng/mL). All cases with a history of

patch use were positive for fentanyl (1-33 ng/mL). The corresponding hair specimens screened positive for 12/13 of these cases with fentanyl levels ranging 55-5120 pg/mg.

Only two individuals with a heroin use history were positive in the blood for fentanyl (7, 24 ng/mL). The corresponding hair was positive at 36 and 1295 pg/mg. The hair of one individual with a heroin use history was positive for fentanyl at 31 pg/mg but the corresponding blood was negative. 6-Acetylmorphine, codeine, morphine, ethanol and cocaine metabolites were identified in blood.

Conclusion: Although the highest concentration of fentanyl in hair and blood occurred in cases with the highest patch dose, there did not appear to be a relationship between blood and corresponding hair concentrations or the dose. The data demonstrated that fentanyl is detectable in hair and may be a useful adjunct to routine specimens in postmortem toxicological analysis.

Fentanyl, Blood, Hair

K65 Postmortem Pediatric Toxicology

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After attending this presentation, attendees will understand the impact of certain toxicological agents in death determination of pediatric patients. There will be imparted many of the reasons why children are not small adults. Through discussion, all attendees will better discern how to interpret toxicological findings in this population.

This presentation will impact the forensic science community by assisting pathologists and toxicologists to integrate pharmaco-/toxicokinetic and pharmaco-/toxicodynamic principles with pathological principles in respect to death determination of children.

In this 9th Annual Special Session within the Toxicology section, pediatric cases involving toxicological findings are discussed. As a relative dearth exists of interpretive information involving toxicological findings in the pediatric population, this session is a forum to help elucidate and clarify such issues. The format is a short case presentation including pharmaco- toxicokinetic data and other relevant ancillary information followed by audience participation to provide interpretive clarity around the case-specific impact of the toxicological findings.

This years presentations will be:

1. Dr. Kenneth Snell, Interim Chief State Medical Examiner, Alabama Dept. of Forensic Sciences, will be discussing the impact of verapamil in a pediatric fatality. Verapamil is a calcium channel blocking agent not generally indicated in children less than 1-year-old. In the current case, a 1 y/o male was found to have verapamil, a non-prescribed medication, in his blood. The i mplication of this finding in the face of a potential inborn error of metabolism will be discussed and presented for audience consult.
2. Dr. Phil Kemp, Chief Toxicologist, OCME, Okalahoma City, OK, will discuss the potential role of methamphetamine on fetal and/or neonatal deaths. Methamphetamine, labeled as the modern day catastrophic drug of abuse, is highly prevalent in the world of drug abuse. Fetal exposure is generally through maternal abuse of the substance. Young children are often exposed through the living environment. Such exposures will be highlighted through case experience where methamphetamine may have been a contributing factor in the determination of death.
3. Dr. Carl Schmidt, Chief Medical Examiner, Wayne County, MI, will review cases of "accidental" fentanyl exposure to young children. A common route of exposure of young children to fentanyl is through manipulation of fentanyl patches. These patches contain specific directives against various forms of manipulation due to exposure to the patch contents. As a drug with a narrow therapeutic index, such exposure is potentially fatal, as will be demonstrated through case history.

Pediatric, Postmortem, Toxicology

K66 Determination of Guanfacine (Tenex) in a Case of Munchhausen by Proxy

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The goal of this presentation is to present a methodology for the determination of Guanfacine (Tenex) and to alert the forensic community that the drug may be used and found in cases of Munchhausen by Proxy.

This presentation will impact the forensic science community by demonstrating the possible treatment of children diagnosed with attention deficit hyperactivity disorder (ADHD) with guanfacine demonstrates the need for a reliable method to detect the drug in cases where Munchhausen by Proxy could be a source of toxicity or unsuspected death.

Guanfacine is a derivative of the nucleic acid guanidine, and is used to treat high blood pressure. Only one published method is available for the quantitation of guanfacine, but the method does not provide for positive qualitative identification. Recently, guanfacine alone or in combination with clonidine has been indicated as a possible treatment for children diagnosed with attention deficit hyperactivity disorder (ADHD).

A four-year-old male child was transferred to an academic tertiary care center for evaluation of a 3-day history of intermittent hyper-somnolence. The child had similar episodes in the past, involving prior hospitalization in the community, an extensive neurological work-up, and follow up with a pediatric neurologist for one year. The child was being treated with valproic acid for possible absence seizures (not seen on prior EEG), and clonidine for sleep. Neurological as well as clinical laboratory testing were all normal, with the exception of a mildly elevated ammonia concentration was resolved without intervention. No drugs were detected on an initial comprehensive drug screen. The child remained hyper-somnolence with intermittent bradycardia without hypotension, while additional neurological studies were negative. Review of the nursing notes revealed that the bradycardic episodes coincided with the somnolent episodes. The child was placed on a three day continuous EEG and camera monitoring. A thorough review of the family history revealed that a sibling was being prescribed guanfacine. Therefore, urine obtained upon admission as well as five other urine specimens were analyzed for guanfacine.

Due to the lack of available mass spectral data, the identification of guanfacine was determined from elicitation of fragmentation ions and pattern. Using a modification of our meperidine/normeperidine urine method, guanfacine was identified and semi-quantified in urine by GC/MS. The pH of 1 ml aliquots of urine calibrators and specimens was adjusted with 0.5 ml of saturated carbonate:bicarbonate solution and extracted using n-butyl chloride with rotate mixing for two minutes, then centrifuged. The upper n-butyl chloride was transferred to a clean test tube, and the guanfacine and the internal standard were derivatized using 50 mL of heptaflurobutyric anhydride (HFBA) at 75 ^{o}C for a minimum of 30 minutes. The n-butyl chloride:HFBA solution was evaporated under nitrogen and reconstituted with ethyl acetate and injected into the GC/MS. Guanfacine was analyzed on a Shimadzu QP-2010 GC/MS system operated in SIM mode, with a DB-5 column (30m x 0.25 mm x 0.33 mm) and a 5 m guard column. The GC oven temperature was programmed from 160 ^{o}C, 0.1 min hold, to 280 ^{o}C at 20 ^{o}C/min. The ions monitored for guanfacine and protriptyline (internal standard) were: *m/z* 86, 272, 274, 159, 161 and 191, 189, respectively. The urine guanfacine concentrations upon initial admission 2, 5, 7 (am), 7 (pm), and 11 days post admission were 3.8, 6.4, 17.9, 3.3, 1.6 mg/L and None Detected, respectively.

The fluctuations in the child's urine guanfacine concentrations correlated with the symptomatic and asymptomatic episodes the child experienced. Upon follow up police interrogation and video, the mother elicited a complete confession. The child dramatically improved, and was eventually discharged.

Guanfacine, GC/MS, Munchhausen by Proxy

K67 Prevalence of Cocaine on Urban and Suburban Elementary Student Desks

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From this presentation, attendees will understand drug contamination in an environment previously unreported in the scientific literature, in this case elementary student desks. Forensic toxicologists, medical review officers, and other forensic investigators will understand contamination factors which may affect their interpretation of drug test results from external matrices. Concerning the learning environments of children, educators tasked with quality improvement will understand issues of drug contamination in public schools and potential problems if widespread drug testing of students were implemented.

This presentation will impact the forensic science community by demonstrating how the limited body of knowledge on which forensic investigators rely when examining surfaces in the workplace and elsewhere, then ascribing evidence of illicit drug use to such results, is supplemented by these findings.

This research tests the hypothesis that surfaces contacted by potentially unsuspecting individuals may be contaminated with significant amounts of cocaine and that this contamination may spread to other areas where drugs are not being used. These findings will supplement the limited body of knowledge on which forensic investigators rely when examining surfaces in the workplace and elsewhere and interpret the significance of the presence of drugs on these surfaces.

Two public schools in the Washington, D.C. area with differing geographic and socioeconomic profiles were chosen for this study. The prevalence of cocaine-related substances in these environments was compared. Researchers obtained permission from the school administrators to swab each student desk in three classrooms per school. Classrooms were at the first, second and fourth grade levels corresponding to student ages of approximately six, seven, and nine-years-old. The entire desk top was swabbed using disposable latex gloves and individually packaged, and sterile, isopropyl alcohol wipes. Negative control swabs were collected from latex gloves before swabbing. Pre-analysis storage temperature was -20°C. In the laboratory, other negative controls were included as additional tests for laboratory contamination. After deuterated internal standards were added to swabs, they were air dried. Analytes were removed from specimens using 0.1 N hydrochloric acid, and then the drugs were extracted using solid phase (SPE) columns. All extracts were analyzed by ion trap CI-GC/MS.

Of 115 inner-city elementary school desks, cocaine was detected on every desk (mean = 150 ng, σ = 140 ng, LOD = 12 ng). In contrast, only two suburban elementary school desks revealed cocaine levels above the LOD (n = 96, mean = 11 ng, σ = 15 ng). All results, including those below the LOD, were included in the statistics. Similarly, the most common cocaine breakdown product, benzoylecgonine, was more prevalent on urban desks (n = 115, mean = 147 ng, σ = 138 ng, LOD = 2 ng) than desks from a suburban school (n = 96, mean = 0.87 ng, σ = 3.3 ng). The ratio of BE to cocaine had no predictive value.

These data reveal quantities of cocaine substances (ng/swab extract) exceeding the limits of detection and limits of quantitation for analyses of other external surface matrices such as hair (expressed in ng/mg) and sweat (expressed in ng/mL extract), as well as inanimate objects, for which there is no mandated standard practice or cutoff for reporting a surface as "positive" for a drug. Because drug use is unlikely in the elementary school environment and by this young age group population, these results support the conclusion that drugs in a real-life environment can transfer from at least one object to human skin and then to another object a distance from the first. These results suggest caution should be exercised when ascribing drug use conclusions based on surface testing when contamination cannot be excluded. BE does not appear to be a good indicator of cocaine use because BE is a congener in street cocaine, because cocaine decomposes to BE in the environment, and because residues containing BE from the sweat of drug users can transfer repeatedly.

Cocaine, Contamination, Children

K1 Analysis of Anabolic Steroids in Urine by LC/MS/MS

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After attending this presentation, attendees will learn of an LC/MS/MS technique for analyzing anabolic substances in urine.

This presentation will impact the forensic community and/or humanity by demonstrating how to quantitate steroids in urine using a non GC/MS technique, which can save additional sample preparation steps including derivatization.

This work represents the use of LC/QQQ mass spectrometry for confirmation of performance-enhancing drugs in urine, targeted for sports doping control analysis. LC/MS/MS with a high-performance 3.5 um rapid resolution column and ionization by APCI on the QQQ instrument, using MRM analysis, provides a lower-cost alternative to the current de-facto standard in international doping control, which is the EI-GC/MS high-resolution magnetic sector instrument. Additionally, increased throughput as a result of bypassing the necessary sample derivatization step, without sacrificing the sensitivity required to meet the minimum required performance levels (MRPLs) of the World Anti-Doping Agency (WADA), is also considered an advantage. Confirmation is carried out using designated quantitation ions in MRM mode. Samples were obtained from the Center for Human Toxicology (University of Utah) to generate calibration curves for quantitation.

The samples were prepared by a liquid/liquid extraction of 3 mL of control urine, spiked at specified levels. The extractions were evaporated to dryness and then reconstituted in 100 □L of liquid chromatographic (LC) mobile phase solvent. The compounds analyzed include 4□-stanozolol, 19-nor-etiocholanolone, tetrahydrogestrinone (THG), and epimetendiol, with internal standards such as methyltestosterone, and d5-etiocholanolone. Calibration curves were generated over concentrations ranging from ½ x to 10 x MRPLs with linearity coefficients (r^2 values) greater than 0.997. Reproducibility at the lowest level (½ x MRPL) was measured in terms of percent relative standard deviation (% RSD) of peak area counts for repeated injections. For triplicate injections the percentage RSDs were typically 1 – 6 %.

The signal-to-noise (S/N) was calculated by first selecting a region of the chromatogram from which to determine the root-mean-squared (RMS) noise, which was then multiplied by a factor of five. The S/N was therefore the height of the peak divided by 5 x RMS noise. This was equivalent to peak-to-peak noise. The limit of detection (LOD) was calculated by first determining the S/N for the peak at the ½ x MRPL and then scaling the concentration down to a level that corresponds to S/N = 3. For example, in the case of epimetendiol, the estimated LOD was 0.05 ng/mL in urine, or 3 pg on-column for a 2 □L injection volume.

LCMS, QQQ, Steroids

K2 The Analysis of Workplace Urine Specimens From Federal Employees Reported as Rejected for Testing

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After attending this presentation, attendees will have a better understanding of the drug and validity characteristics of Federal employee urine specimens that were not tested & reported as Rejected for Testing.

This presentation will impact the forensic community and/or humanity informing the forensic community with objective data for Federal employee workplace urine drug testing specimens reported as Rejected for Testing at two SAMHSA certified laboratories and allow the re-evaluation of minimum drug testing volume requirements.

Introduction: Anecdotal comments infer that the incidence of drug positivity and/or unacceptable specimen validity in specimens that would be routinely reported as Rejected for Testing, mostly due to volume less than 30 mL, is considerably higher than in those specimens that had sufficient volume to routinely test. These anecdotes apply generally to workplace urine drug testing and also specifically to specimens tested under Federal authority.

Objective: To determine if Federal employee specimens that were reported as Rejected for Testing by SAMHSA certified laboratories provided similar drug positive, adulterated, invalid, or substituted (non-negative) results when compared to the results of other federally regulated specimens that were tested and reported over the same time frame in SAMHSA certified laboratories.

Methods: Specimens submitted through the Federal employee drug testing program and reported as Rejected for Testing were obtained from two SAMHSA certified laboratories, with no way to link the specimens to donors. The specimens (both A and B bottles, when available) were tested with a Microgenics MGC240 using Microgenics DRI (Cannabinoid Metabolites, Cocaine Metabolite, Phencyclidine, Opiates, Amphetamines), Microgenics Detect (Creatinine, pH) and Axiom (Oxidant) assays. Those specimens for which drug tests were presumptively positive by immunoassay and for which validity tests were not within the acceptable range as required in the Mandatory Guidelines (69 Fed. Reg. 19644, effective Nov. 1, 2004) were sent to a reference laboratory for confirmatory drug tests by GC/MS and necessary validity testing in accordance with those Mandatory Guidelines.

Results: Specimens from 478 donors that had been reported as Rejected for Testing from November 2004 through April 2006 with a volume of at least 5 mL were tested. Of these 478 donor specimens, 63 donors provided specimens with either presumptive drug positive results and/or unacceptable validity test results, including dilute. Of these 63 donor specimens, 11 donors provided drug negative dilute specimens that were not tested further for drugs below the Mandatory Guidelines cutoffs, 45 donors provided specimens having a single presumptive drug positive result or unacceptable validity test results. Confirmatory testing of these 45 specimens yielded the following results:

	AMP	BZE	OPI	PCP	THCA	ADULT	INV	SUBS	Total
Screened (+)	14	4	6	0	6	0	15	0	45
Confirmed (+)	8	4	6	NA	6	NA	15	NA	39

Of these 63 donor specimens, seven donors provided specimens with multiple results: multiple presumptive drug positives, multiple unacceptable validity test results, or a combination of presumptive drug positive and unacceptable validity test results. Confirmatory testing of these seven specimens yielded the following results:

Positive Screening Test	ADULT/ INV	AMP/ BZE	AMP/ OPI	AMP/ THCA	BZE/ DIL	BZE/ THCA	OPI/ THCA
Confirmatory Test Results	pH too high	AMP MAMP	NEG AMP NEG MAMP	AMP MAMP	BZE	BZE	NEG MOR NEG COD
	Abnormal Creat&SpGr	BZE	COD MOR	THCA	DIL	THCA	THCA

Only 13 of the 478 specimens reported as Rejected for Testing were collected under Post-Accident or Reasonable Suspicion/Cause conditions. One of those 13 donors provided a dilute specimen. Specimens from three of these 13 donors (23.1%) provided drug positive results: two donors were positive for THCA, and one donor was positive for both BZE and THCA. Of the specimens provided by the 478 donors, 9.6 % (46) provided specimens with drug positive and/or unacceptable specimen validity results (not including the 11 dilute specimens) as compared to 2.2% of all Federal & federally regulated specimens tested and reported by all SAMHSA certified laboratories during the same time period that were also tested for drugs and specimen validity (not including dilute results).

Conclusions: Of the 478 specimens reported as Rejected for Testing, 353 were rejected for insufficient volume (< approximately 30mL), with only 18 of these 353 specimens having leaked in transit resulting in their insufficient volume. This suggests that the minimum volume requirement for specimen rejection may need to be re-evaluated.

Rejected Specimens, Federal Employees, Non-Negative Incidence

K3 Alcohol, Drugs, and Homicide

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After attending this presentation, attendees will better understand the role of alcohol and drug abuse in violent deaths.

This presentation will impact the forensic community and/or humanity by providing additional support that alcohol and drug users are at greatly increased risk of being victims of homicide.

In the United States, homicide is the third leading cause of death for persons 15-24 years of age and the leading cause of death for black males 15-34 years of age. It has been estimated that one out of ten homicide victims are drug-related.

A retrospective study of homicide victims in the State of Maryland was conducted for 2003, 2004, and 2005 to determine alcohol and/or illicit drugs use among homicide victims. During the past three years, a total of 1,674 homicides occurred in Maryland, with an average rate of 10.1 homicides per 100, 000 individuals. Males were six times more likely to become homicide victims (85.7%) than were females (14.3%). Blacks were 5.9 times more likely than whites to be victimized. Black males were at the greatest risk, to become victims. Of homicide victims, 71 % (1187/1674) were black males, with an average rate of 53.5 per 100,000 populations.

Comprehensive alcohol and drug testing were performed on all of the homicide victims. Alcohol and /or drugs were found in a significant portion of homicide victims. Of this group, 739 out of 1,674 homicide victims (44%) showed some form of recent illicit drug and/or alcohol use. Alcohol was positive in 33% of the cases. Among illicit drugs, cocaine was the most common drug detected in the homicide victims (15%), followed by Narcotics (6%), Phencyclidine (PCP) (3%), and Methylenedioxymethamphetamine (MDMA) (1%). More male victims (46%) were positive for alcohol and/or drugs than were female victims (22%). Blacks (45%) were slightly more likely than whites (41%) to be positive for alcohol and/or drugs.

The most common cause of homicide in Maryland was firearm injury (77%), followed by sharp force injury (10%), blunt force injury (8%), suffocation/strangulation (2%). Among victims caused by firearm injury, 34% were positive for alcohol, 10% for cocaine, 7% for narcotics, 4% for PCP, and 1% for MDMA. The victims from sharp force injury were 33% positive for alcohol, 7% for cocaine, 2% for narcotics, and 1% for PCP. Alcohol, cocaine, and narcotics were detected in 25%, 6%, and 6% respectively among victims of blunt force injury, and in 36%, 3%, and 11% respectively among the victims from suffocation/strangulation.

Forensic Toxicology, Drugs, Homicide

K4 Gamma-Hydroxybutyrate (GHB) - Withdrawal With Severe Rhabdomyolysis, Hyperkalemia, and Cardiac Arrest

David M. Benjamin, PhD, 77 Florence Street, Suite 107, Chestnut Hill, MA 02467*

After attending this presentation, attendees will learn about the presentation of severe GHB withdrawal.

This presentation will impact the forensic community and/or humanity by informing the forensic community that severe GHB toxicity leading to death can occur in the absence of proper treatment.

Gamma-Hydroxybutyrate (GHB) is well known to the forensic toxicology community for its euphoric, soporific, and intoxicating properties. Fatalities have occurred from recreational use of GHB at clubs or "Raves," and when GHB has been added surreptitiously to anther person's beverage, usually to facilitate a sexual assault (Drug Facilitated Sexual Assault, DFSA). However, chronic use of GHB produces a dependence similar to that of ethanol, and a withdrawal syndrome very similar to ethanol-related delirium tremens (DTs) has been reported in the literature.

In this case, the decedent was a 28-year-old a male with a long-term, high dose dependence on GHB. In order to ensure his supply of GHB, the man distributed GHB. At the time of his cardiac arrest, the decedent was in police custody for possession and trafficking of GHB. During the booking process, the decedent complained of symptoms of GHB withdrawal. Over a further two-day period of abstinence, the decedent became disoriented, agitated, began to hallucinate and injure himself. To prevent further injury, he was restrained in a "pro-straint" chair with restraints at the wrists, chest, and ankles. The next day, he was less combative but continued to have hallucinations and exhibit rambling speech. After 22.5 hours, he was released from restraints and transferred to a soft-walled cell equipped with video monitoring. He subsequently was observed walking around the cell, but by the end of the second hour, he was observed slumped in the corner of his cell. Guards entered the cell and found him unresponsive, pulse less and apneic. EMS personnel were summoned and also reported him as pulse less and apneic, with a cardiac monitor indicating pulseless sinus tachycardia. CPR was started, the patient was intubated and given naloxone and dopamine, and he was transported to the hospital emergency room (ER).

In the ER, the patient was initially hypotensive but his blood pressure stabilized and dopamine was tapered and stopped. A CAT scan of the head and chest x-ray were normal. However, serum potassium was 8.0 mEq/L, urea nitrogen was 97, serum creatinine was 4.1 and creatine phosphokinase (CPK) was 75,000 IU/L with a heart muscle fraction (CK-MB) of 0.3%. Serum troponin was negative. Urine drug screening was negative for ethanol, salicylates, phencyclidine, cocaine, amphetamines, cannabinoids, opiates, barbiturates, and tricyclic antidepressants. Urine drug screening was positive for benzodiazepines. The admitting MD's assessment was cardiac arrest, probably secondary to acute hyperkalemia and secondary to acute renal failure secondary to rhabdomyolysis secondary to polydrug abuse; liver failure; coagulopathy; and respiratory failure. The patient's clinical course worsened. He became increasingly anuric and his CPK continued to rise along with liver function tests. Four days after admission to the ER, a follow up CAT scan of the head showed diffuse cerebral edema with herniation which led to his being declared brain dead. Autopsy findings included anoxic encephalopathy, cerebral edema, herniation, and necrosis of the cerebellar tonsils, secondary compression, and necrosis of the cervical spine; rhabdomyolysis with necrosis of skeletal muscle cells; acute renal failure with acute renal tubular necrosis of kidneys; bronchopneumonia, and centrilobular necrosis of the liver (shock liver). The cause of death was listed as anoxic encephalopathy following resuscitation from cardiac arrest due to GHB withdrawal syndrome, and the manner of death was listed as natural. The Medical Examiner offered the following comment in his report: "The suddenness of collapse and development of rhabdomyolysis are suggestive of seizures as the mechanism of cardiac arrest during withdrawal in this case." Neither the record of incarceration nor video monitoring included any reference to a witnessed seizure. There were no bite marks on the tongue. The ME was on the right track, but has not quite accounted for the markedly elevated CPK and muscle death which led to the liberation of sufficient intracellular potassium to raise normal serum potassium levels (3.5-5.3 mEq/L) up to 8.0 mEq/L and cause a cardiac arrest.

The key to understanding this case was the development of rhabdomyolysis with the associated release of myoglobin that got trapped in the kidneys' glomeruli and caused renal failure which potentiates the hyperkalemia caused by release of potassium from intracellular sites in skeletal muscle cells. While a seizure can cause both hyperpyrexia and physical damage to muscle cells, the order of magnitude of the cell death and associated hyperkalemia that led to this patient's death indicated massive muscle death and release of sufficient potassium to raise serum potassium approximately 3 mEq/L. A better explanation for the massive tissue damage would be the hyperpyrexia and dehydration generated from agitation and "fighting with the restraints." Elevated catecholamine and potassium levels are often cited as possible physiological triggers; however, GHB can also cause DTs similar to those of ethanol withdrawal, which include autonomic hyperactivity and typically occur 72-96 hours following abstinence.

Gamma-Hydroxybutyrate (GHB) Withdrawal, Rhadbomyolysis, Hyperkalemia

K5 Recent Paramethoxymethamphetamine (PMMA) Deaths in Taiwan

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After attending this presentation, attendees will learn about: 1) the action of PMMA; 2) a sensitive GC/MS method for the analysis of PMMA; and 3) the concentration of PMA and PMMA in postmortem specimens.

Trafficking of PMA and PMMA tablets are found in Taiwan from December 2005 to March 2006. Five deaths caused by acute toxicity of PMA and PMMA in April have posed a threat to the society in Taiwan because people are unaware that PMMA in combination with PMA are sold as Ecstasy. PMA is classified as Schedule II controlled drug but PMMA has not been classified as a controlled substance in Taiwan. This presentation will impact the forensic community and/or humanity by presenting the need to bring PMMA as Scheduled II controlled drugs and also to impose criminal penalties through legislation.

Paramethoxyamphetamine (PMA) and paramethoxymethamphetamine (PMMA) are methoxylated phenylethylamine derivatives that have been banned in Taiwan since December 2005. Case history and pathological and toxicological findings of eight recent PMMA fatalities were investigated. All specimens from these cases were initially identified by AxSYM fluorescence polarization immunoassay screening test for amphetamines with a 300 ng/mL cutoff. Specimens screened positive were confirmed and quantitated by gas chromatography-mass spectrometry. The mean age of these PMMA-related fatalities was 18.9 ± 4.4 years ranging from 14–25. Seven (87.5%) of these eight cases were men. The mean, standard deviation, and range of PMA found in the heart blood collected from these eight cases were 0.213, 0.144, and 0.079–0.489 µg/mL; the corresponding data for PMMA were 4.312, 4.806, and 1.208–15.824 µg/mL, respectively. Other drugs, such as MDA, MDMA, ketamine, norketamine, hydroxymidazolam, methamphetamine, and pentobarbital, were also found in these cases. This paper describes five cases of fatal overdose from PMMA ingestion that occurred in April 2006 in Taiwan. These cases reflect the well-known fact that street drugs offered as ecstasy pills do not necessarily contain MDMA, but frequently differ in composition even if they have the same logo. Users of these pills therefore always take the risk of consuming pills with dangerous life-threatening ingredients.

Forensic Toxicology, PMMA Deaths, Drug of Abuse

Table I. Postmortem Distribution of PMA and PMMA (μg/mL) Found in Specimens Collected from Eight Fatal Cases in Taiwan.

Case	Age	Sex	Drug Ratio	Heart Blood	Urine	Other Drugs (Blood, μg/mL)	Cause of Death	Manner of Death
1	14	M	PMA	0.145	6.263	Ketamine, 0.018	PMMA	Accidental
			PMMA	3.017	157.51	Norketamine, 0.017	intoxication	
			PMA/PMMA	0.048	0.040			
2	15	M	PMA	0.196	1.808	ND[a]	PMMA	Accidental
			PMMA	1.554	7.646		intoxication	
			PMA/PMMA	0.126	0.237			
3	18	F	PMA	0.367	—[a]	Ketamine, 1.210	PMMA	Suicide
			PMMA	15.824	—	Norketamine, 0.558	intoxication	
			PMA/PMMA	0.023	NA[a]	Methamphetamine, 0.139		
4	19	M	PMA	0.489	—	Pentobarbital, 0.283	PMMA	Accidental
			PMMA	4.014	—	intoxication		
			PMA/PMMA	0.122	NA			
5	25	M	PMA	0.122	0.379	MDMA, 0.199	PMMA	Accidental
			PMMA	1.208	11.857	Ketamine, 0.154	intoxication	
			PMA/PMMA	0.101	0.032	Norketamine, 0.177		
6	14	M	PMA	0.205	5.964	Methamphetamine, 0.109	Multiple-drug	Accidental
			PMMA	2.193	88.706	MDA, 0.134	intoxication	
			PMA/PMMA	0.093	0.067	MDMA, 14.637		
						Ketamine, 0.199		
						Norketamine, 0.326		
7	22	M	PMA	0.097	0.263	Ketamine, 0.041	PMMA	Accidental
			PMMA	1.969	14.208		intoxication	
			PMA/PMMA	0.049	0.019			
8	24	M	PMA	0.079	—	MDA, 0.172	Multiple-drug	Accidental
			PMMA	4.718	—	MDMA, 4.322	intoxication	
			PMA/PMMA	0.017	NA			

[a] ND: Not detected; NA: Not applicable; —: Sample (or information) not available.

K6 Cocaine Impaired Driving: Evaluation of Toxicology, Driving Behavior, and Impairment Symptoms in Arrested Drivers

Rachael Malfer, Anna Leggett, BS, Sharla McCloskey, BS, and Sarah Kerrigan, PhD, Forensic Science Program, College of Criminal Justice, Sam Houston State University, Chemistry and Forensic Science Building, 1003 Bowers Boulevard, Huntsville, TX 77341*

The goal of this presentation is to evaluate common observations, driving behavior, and impairment symptoms in actual drivers that have used cocaine and are apprehended for driving while intoxicated (DWI).

This presentation will impact the forensic community and/or humanity by assisting with the toxicological interpretation of cases by comparing common signs, symptoms, observations and driving behavior in drivers suspected of driving under the influence of cocaine.

Driving behavior, reason for the traffic stop, documented signs and symptoms and quantitative blood toxicology are compared in a series of 48 persons suspected of driving under the influence of cocaine.

Cocaine is a central nervous system stimulant, which at high doses can produce characteristic physiological and behavioral effects that are inconsistent with safe driving. However, many scientific studies are limited by the low dose of drug that is administered to human subjects, doses that typically much lower than those used by illicit drug users. Multiple drug use, tolerance, dependence, and withdrawal effects of the drug make interpretation of these cases challenging. In many instances, toxicologists take a case-by-case approach to impairment by drugs other than alcohol. This process involves a careful review of toxicology test results, driving and observations that were made by law enforcement personnel.

In this series of 48 drivers, only 10 cases involved cocaine alone. The remaining 38 cases involved multiple drug use, most frequently ethanol, marijuana, benzodiazepines, and methadone. Mean, median and mode cocaine concentrations were 0.09 ± 0.12 (SD), 0.05 and 0.02 mg/L respectively. Mean, median and mode benzoylecgonine (BE) concentrations were 0.81 ± 0.94 (SD), 0.43 and 0.14 mg/L respectively. The total range of concentrations for cocaine and BE for all the cases (n=48) were 0.01-0.53 and 0.03-4.10 mg/L respectively. Comparison of quantitative drug results for cocaine only and cocaine in combination with other drugs indicated no significant differences. The most common reason for the traffic stop was a crash. Other common reasons for the stop were notification by dispatch (following a report of impaired driver from the public) and impaired speed control. The performance on standardized field sobriety tests (SFSTs), which can provide important information on mental and physical function, were evaluated for all the cases. SFSTs were not performed in every case due to injuries sustained in a crash, uncooperative subjects, or subjects that were too impaired to

* Presenting Author

safely perform the tests. Documented signs, symptoms, and observations made by the arresting officer were compared. The most common observation for both cocaine only and cocaine in combination with other drugs was impaired psychomotor function.

Cocaine, Impaired, Driving

K7 Driving Under the Influence of Methamphetamine: Comparison of Driving Behavior and Impairment Symptoms in Subjects Arrested for Driving While Intoxicated (DWI)

Sharla McCloskey, BS, Anna Leggett, BS, Rachael Malfer, and Sarah Kerrigan, PhD, Sam Houston State University, College of Criminal Justice, 1003 Bowers Boulevard, Huntsville, TX 77341*

The goal of this presentation is to review driving behaviors and impairment symptoms in a series of sixty drivers suspected of driving under the influence of methamphetamine.

This presentation will impact the forensic community and/or humanity by assisting with the toxicological interpretation of cases by comparing common signs, symptoms, observations and driving behavior in drivers suspected of driving under the influence of methamphetamine.

In drug impaired driving cases, a toxicologist usually requires information from a variety of sources before they provide an opinion regarding possible impairment. Information in the police report, such as the reason for the stop, performance on the standardized field sobriety tests and other observations made by law enforcement or witnesses at the scene can be of interpretive value. A series of sixty drivers who used methamphetamine and were subsequently arrested for driving under the influence of drugs (DUID) are presented.

Methamphetamine is a central nervous system stimulant that can produce wide-ranging effects, depending on the dose and phase of use. At high doses methamphetamine has the potential to impair skills that are important for safe driving. Quantitative blood drug results are valuable from a toxicological standpoint but must be interpreted within the context of the case. Low concentrations of the drug in the later phase of drug use (down-side) can produce effects that are detrimental to safe driving. Multiple drug use, tolerance, dependence, and withdrawal effects of the drug make interpretation of these cases challenging.

In this series of 60 drivers arrested for DWI, quantitative blood methamphetamine concentrations were reviewed, together with the reason for the stop, signs, symptoms, performance on standardized field sobriety tests, and other observations. Cases involving only methamphetamine were compared with methamphetamine in combination with other drugs. Mean, median and mode blood methamphetamine concentrations were 0.3, 0.2 and 0.1 mg/L (n=60) respectively. Amphetamine was detected in blood samples in 42 of the cases and reported quantitatively in 28. Concentration ranges for methamphetamine and amphetamine were 0.01-3.20 and 0.02-0.26 mg/L respectively. The reason for the stop, performance on field sobriety tests and roadside observations were compared for different sub-sets of data that were organized by concentration and drugs present. The most common reasons for the traffic stop were a crash (n=18), equipment violation (n=13), erratic driving (n=9) and notification by dispatch (n=8). The performance on standardized field sobriety tests were evaluated in terms of the number of clues, together with other observations, the most common of which were bloodshot eyes and impaired balance and coordination. Individual cases are presented to illustrate some of the common interpretive challenges including tolerance, withdrawal and the phase of drug use within the context of quantitative drug toxicology.

Methamphetamine, Impaired, Driving

K8 Driving Behavior and Impairment Symptoms in Cannabinoid Positive Subjects Arrested for Driving Under the Influence of Drugs (DUID)

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The goal of this presentation is to review driving behaviors and impairment symptoms in a series of 108 cannabinoid positive drivers suspected of driving under the influence of drugs.

This presentation will impact the forensic community and/or humanity by assisting with the toxicological interpretation of cases by comparing common signs, symptoms, observations and driving behavior in drivers suspected of driving under the influence of marijuana.

Quantitative drug toxicology is complemented by case specific observations, such as performance on field sobriety tests, signs, symptoms and other observations made by qualified law enforcement personnel. Quantitative blood drug results and supplemental information are presented in a series of 108 cannabinoid positive drivers.

Marijuana can produce a unique spectrum of effects that prevents classification into only one class. From an impaired driving standpoint however, scientific studies have shown that delta-9-tetrahydrocannabinol (THC) can impair cognitive and psychomotor functions associated with driving in a dose dependent manner. Yet, there is no widely accepted concentration of THC in blood at which a driver is deemed impaired for the purposes of driving. Quantitative blood toxicology is important in DUID cases involving cannabinoids, but must be carefully interpreted within the context of case specific information and the collection time, due to the rapid decline of THC in blood following smoking. Interpretation is further complicated by the frequency of multiple drug use among impaired drivers, particularly those using cannabinoids.

In a series of 108 drivers arrested for DWI, quantitative blood cannabinoid concentrations were reviewed, together with the reason for the stop, signs, symptoms, performance on standardized field sobriety tests, and other observations. Cases involving only cannabinoids were compared with cannabinoids in combination with other drugs. Mean, median and mode 11-nor-9-carboxy-delta-9-THC (carboxy-THC) concentrations were 39, 29 and 7 ng/mL (n=108) respectively. THC was detected in 50 of the cases and reported quantitatively in 45, with a mean, median, and mode of 5, 4 and 3 ng/mL respectively. Concentration ranges for THC and carboxy-THC were 2-18 and 2-235 ng/mL respectively. The limit of quantitation of the method was 2 ng/mL. The reason for the stop, performance on field sobriety tests and roadside observations were compared for different sub-sets of data that were organized by presence of parent drug (THC) and other drugs present. The three most common reasons for the traffic stop were speeding (n=30), crash (n=25) and weaving (n=21). The performance on standardized field sobriety tests were evaluated in terms of the number of clues, together with other observations, the most common of which were impaired balance and coordination (n=54), bloodshot eyes (n=50), watery/glassy eyes (n=32) and slurred or thick speech (n=26).

Marijuana, Impaired, Driving

K9 Fatal Ephedrine Intoxication in a Chronic Ephedrine User Who Had Cardiovascular Disease

Diana G. Wilkins, PhD, Center for Human Toxicology, Biomed Research Polymers Building, Room 490, Salt Lake City, UT 84112; Ling Li, MD, and David R. Fowler, MD, Office of the Chief Medical Examiner State of Maryland, 111 Penn Street, Baltimore, MD 21201; Brienne Brown, MS, Center for Human Toxicology, Biomed Research Polymers Building, Room 490, Salt Lake City, UT 84112; and Stephen J. Kish, PhD, Centre Addiction Mental Health, 250 College Street, Toronto, Ontario M5T 1R8, Canada*

After attending this presentation, attendees can be expected to enhance their understanding of the possible risk factors for fatal ephedrine intoxication.

This presentation will impact the forensic community and/or humanity by providing additional information to the public indicating a possible risk factor associated with the use of a drug commonly found in dietary supplements.

Introduction: Ephedrine is an alkaloid present in some dietary supplements which has been widely used for body weight reduction and energy enhancement. Although serious adverse reactions have been described in the literature, there is still some controversy over the prevalence of such adverse events and the factors that increase the risk to users of the drug. This presentation describes a fatal ephedrine intoxication in a subject who had arteriosclerotic cardiovascular disease.

Case Study: A 40-year-old male Caucasian was found unconscious and a resuscitative attempt was unsuccessful. The decedent was reported to have used the non-prescription drug "MaxAlert" that purportedly contains 25 mg ephedrine hydrochloride and 100 mg guaifenesin per tablet [1].

An autopsy, conducted approximately 9 hours postmortem, revealed arteriosclerotic cardiovascular disease with the left anterior descending artery 60% occluded with plaque and the right coronary artery 100% occluded. The heart weighed 550 grams. Examination of other organs, including neuropathological analysis of the brain, was unremarkable. Toxicological analysis of cardiac blood was negative for alcohol and disclosed the presence of ephedrine (10.0 mg/L) and phenylpropanolamine (0.8 mg/L). The urine tested positive for ephedrine and phenylpropanolamine.

Toxicological results of autopsied brain and scalp hair: Hair strands, cut close to the scalp, were individually aligned (root-to-tip) and segmented into one inch segments. The resulting three segments were sequentially washed with 3 x 1 mL 1% SDS, 3 x 3 mL MilliQ water, 3 x 3 mL methanol [2]. Deuterated internal standards were added to 20-mg hair, followed by addition of 2 mLs 0.1 N HCl, and subsequent overnight incubation at 37° C. Specimens were buffered to pH 5.5, extracted with a solid-phase procedure, and screened for a panel of selected drugs by liquid chromatography (LC) atmospheric pressure ionization - electrospray (API - ES) mass spectrometry (MS). Ephedrine and methamphetamine, respectively, were detected at the following concentrations: Segment #1, 34.3, and 1.6 ng/mg; Segment #2, 35.9 and 1.8 ng/mg; Segment #3, 41.9 and 1.6 ng/mg hair.

Tissue homogenates (occipital cortex) were also prepared, deuterated standards added, and specimens immediately extracted using the extraction and MS procedures described above with minor modifications. Ephedrine alone was detected in brain at 10.2 ng/mg tissue.

Discussion: The results of the toxicological analyses indicate that the subject used ephedrine both acutely (blood and brain drug positive) and chronically (hair segments drug positive). In addition, the analyses disclosed evidence of some chronic, but not acute, exposure to methamphetamine.

It was proposed at the 2004 AAFS workshop on ephedrine that toxicity to the drug might commonly occur in asymptomatic individuals who have an undiagnosed underlying disease. The findings of the case study are consistent with this possibility as the autopsy disclosed severe cardiovascular disease, a condition that would be expected to predispose the drug user to complications arising from the sympathomimetic property of ephedrine. Nevertheless, the alternate possibility has to be considered that the high concentration of ephedrine found in the deceased could have been sufficient, on its own, to have caused death. In this regard, the concentrations of ephedrine found in blood and brain of this case are similar to those reported in a fatal ephedrine intoxication in which no underlying pathology could be observed at autopsy [3].

The final cause of death was ruled for this case to be ephedrine intoxication and arteriosclerotic heart disease.

Conclusion: The case study finding provides additional support to the proposal that fatal ephedrine intoxication can occur in a subject having underlying cardiovascular disease.

References:
[1] http://www.fda.gov/foi/warning_letters/d1218b.pdf
[2] *J Forensic Sci* 2004;49:1106-12.
[3] *J Forensic Sci* 1997;42:157-9.

Ephedrine, Cardiovascular, Fatality

K10 Intoxilyzer® 8000 Stability Study

John J. Kucmanic, BS, Ohio Department of Health, 161 South High Street, Akron, OH 44308*

After attending this presentation, attendees will gain valuable information about the stability and performance of one model of breath alcohol instrumentation.

This presentation will impact the forensic community and/or humanity by adding additional analytical validity to the alcohol results reported from the Inxoxilyzer® 8000 which will aid state programs and prosecutors in convicting drunk drivers.

This study was conducted to investigate the performance and stability of the Intoxilyzer® 8000 operated in either the Alternating Current (AC) or Direct Current Battery (DC_{BT}) mode over a period of one year when the light source remained illuminated.

During this testing period, the light source remained on for 341 days which amounted to greater than 8,184 hours of source/detector life. Furthermore, the instrument detector voltages for both filters were documented for over 240 days to monitor the stability of light source over the evaluation period.

Instrument checks were performed at random intervals to verify the accuracy and precision of the instrument. Each testing day 20 consecutive instrument checks were run in the AC mode followed by 20 consecutive tests using DC_{BT} mode. A total of 4,820 tests were performed on one Intoxilyzer® 8000 using an Instrument check solution lot with a target value of 0.099 g/210L. The statistical mean, median, and mode derived from the analysis of all tests were identical to the target value.

The Intoxilyzer® 8000 pulsed light source/detector is robust and over a period of one year of operation was able to produce acceptable results for the instrument check solution tested in either the AC or DC_{BT} mode with no appreciable loss of calibration or precision.

Intoxilyzer® 8000, Alcohol, Breath Testing

K11 Simultaneous Screening and Confirmation of Drugs in Biological Fluids Utilizing LC/MS/MS

Tania A. Sasaki, PhD, Applied Biosystems, Inc., 850 Lincoln Centre Drive, MS 430, Foster City, CA 94404*

After attending this presentation, attendees will understand a simple method used to detect and confirm the presence of drugs of abuse and their metabolites in various biological matrices. This method has very simple sample preparation and can detect and identify drugs across several different compound classes.

This presentation will impact the forensic community and/or humanity by demonstrating the ability of toxicologists to screen samples in a simpler and quicker manner. They also will have the capability to screen across several drug classes in a single experiment.

Rapid detection, identification, and quantification of drugs in biological matrices are important aspects of forensic toxicology. Typically, GCMS, HPLC, immunoassays, TLC, and various other methods are used to screen for drugs and GC/MS is used confirmation of drugs in Forensic analysis. The use of LC/MS/MS for screening, confirmation, and quantitation of drugs in toxicological assays is becoming increasingly common due to the simplicity, selectivity, and sensitivity of the technique.

A simple LC/MS/MS method was developed to analyze biological fluids (urine, blood, and oral fluids) for hundreds of common drugs of abuse and/or their metabolites, including opioids (including Fentanyl), sympathomemetic amines, antidepressants, benzodiazepines, cocaine, and THC. A hybrid triple quadrupole/linear ion trap mass spectrometer was used for detection, which allowed confirmation using full scan MS/MS spectra and quantitation using multiple reaction monitoring (MRM). Detection limits for all analytes can be as low as pg/mL range. Sample preparation was eliminated or greatly simplified versus analogous experiments using other chromatographic techniques and experimental run times were on the order of 10 - 15 minutes.

Toxicology, Drug Screening, LC/MS/MS

K12 A Novel Method to Extend the Detection Window of Drug Administration in Victims of Malignant Assault With Hybrid LC/MS/MS Technology Combining Triple Quadrupole and Ion Trap Technology

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After attending this presentation, attendees will understand the advantages of using hybrid triple quadrupole linear ion trap mass spectrometry to identify phase I and phase II metabolites of drugs.

This presentation will impact the forensic community and/or humanity by helping to determine whether a drug has been administered, even after the parent drug has been completely eliminated from the victim's body.

A research method has been developed to detect drug intake long after a dose has been administered. This is achieved by detecting specific Phase I and Phase II metabolites that are continually excreted post dose, far longer than the parent drug. Drugs and metabolites are detected in positive mode utilizing specific Multiple Reaction Monitoring (MRM) experiments. Information-dependent criteria for acquisition of an enhanced product ion (EPI) scan result in precursor ion fragmentation to characteristic product ions. Fragmentation occurs at varying collision energies and enables spectral comparison to drug libraries. In addition, Phase II metabolites, namely glucuronides are detected using true Neutral Loss (NL) scanning and identified by EPI acquisition and spectral matching. The loss of dehydroglucuronic acid with a m/z ratio of 176 is characteristic of all glucuronide metabolites. Chromatographic separation is based on a 2.1 mm ID, 5 micron Gemini column with an acetonitrile, formic acid, and ammonium formate mobile phase gradient ramp optimized for separation of various drugs and metabolites. The method is used to detect drugs in forensic and clinical research samples and was developed to provide greater scope, sensitivity, and selectivity compared to conventional methods of drug detection. The method will help to determine whether or not a drug has been taken/administered even after the parent drug has been completely eliminated from the body of the victim.

LC/MS/MS, Metabolites, Toxicology

K13 Application of Ion Mobility Spectrometry to the Analysis of Gamma-Hydroxybutyrate and Gamma-Hydroxyvalerate in Toxicological Matrices

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After attending this presentation, attendees will learn about a rapid, portable, screening technique for the simultaneous analysis of GHB, GHV, and analogs in urine. The physical extraction of the hydrophilic analytes from urine will be discussed, as will the benefits of ion mobility spectrometry in forensic analyses.

This presentation will impact the forensic community and/or humanity by introducing a physical extraction with ion mobility spectrometry as a rapid, portable screening technique suitable for the detection of GHB, GHV, and analogs in urine.

The predator drug, gamma-hydroxybutyrate (GHB), the lactone precursor (gamma-butyrolactone, GBL), and the diol precursor (1,4-butanediol, BD) continue to present significant analytical challenges to forensic toxicologists and chemists. The five-carbon analog (gamma-hydroxyvalerate, GHV) and the corresponding lactone (gamma-valerolactone, GVL) are emerging as substitutes for GHB, adding further complications.

A rapid and reliable screening test for detection of GHB and GHV would be useful for toxicologists as well as forensic chemists working with solid dose samples. This lab has previously reported a microcrystal test effective for aqueous solutions, but felt the development of a rapid, simple instrumental test effective for screening urine required development. In addition, GHB and GHV are extremely hydroscopic and hydrophilic, negating the possibility of rapid and simple extractions that might be necessary for quick screening. Thus, any successful GHB/GHV screening methodology must either be matrix independent or insensitive or capable of rapid and semi-quantitative extraction from the matrix. The latter issue has been the limiting factor to date.

Ion mobility spectrometry (IMS) was investigated as a method of screening urine for the presence of these drugs and their degradation products. In the present study, a high-performance split/splitless injector and autosampler were utilized to effect a physical separation of GHB and GHV from aqueous matrices (including urine) based on differences in relative volatility. This was achieved by a timed period of solvent evaporation followed by rapid temperature increase and thermal desorption of the residuals. The injection method in effect replaces problematic solvent extraction methods with a physical extraction, an efficient method in the present case considering the hydrophilic nature of GHB. Sample was introduced directly into a detection system

without any chromatography, resulting in rapid analysis times. The negative ion mode showed the greatest sensitivity with detection limits in the low parts-per-million range for GHB and GHV. Since GHB is often delivered in alcoholic beverages, ethanol and acetaldehyde, along with potential interfering compounds methanol, isopropanol, acetone, were also analyzed. None were found to interfere. The thermally-induced ring opening prevented differentiation of GHB and GBL using direct injection/ thermal desorption protocol, but IMS does show promise as a rapid, simple, and affordable screening technique for GHB and related compounds.

Reduced mobilities of GHB, GHV, GBL, GVL, and BD were determined by analysis of vapor generated from neat samples. Resulting Ko's are shown in Table 1. GHB, GBL, and BD were indistinguishable based on Ko's and standard IMS alarm variability (standard is ±50us in the drift times). Very slight difference in the reduced mobilities of GHB and GBL were noted, consistent with earlier results.

Table 1

Analyte	GHB	GHV	GBL	GVL	BD
Reduced Mobility (K_o)	1.7097	1.6190	1.7105	1.6380	1.7103

To gauge applicability in toxicology, GHB and GHV were dissolved in saturated synthetic urine solutions followed by serial dilutions as described previously. The synthetic urine was found to have no interfering peaks and LOD was estimated to be in the low ppm range by serial dilution methods. Furthermore, GHB and GHV are distinguishable in synthetic urine. Although the urine matrix contributes additional background peaks, the analytical peaks remain discernible.

Ion Mobility Spectrometry, GHB, GHV

K14 Chromatographic and Mass Spectrometric Characteristics of Multiply Derivatized Opiates

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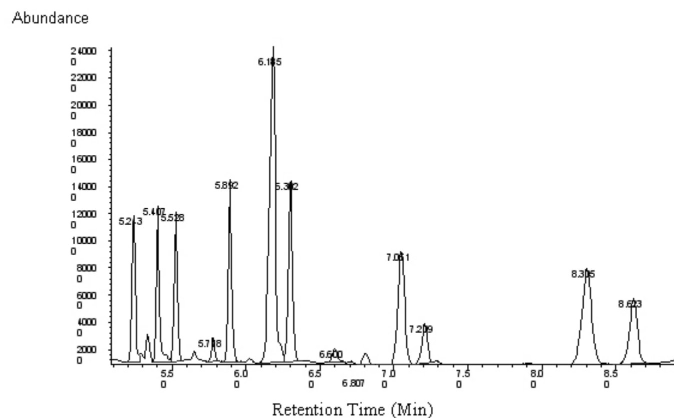
After attending this presentation, attendees will have deeper appreciation on how the analysis of drugs/metabolites in biological media can be facilitated by various chemical derivatization methods.

This presentation will impact the forensic community and/or humanity by illustrating how multiple derivatization approaches can facilitate chromatographic resolution of structurally closely related opiates (see the list below), allowing a single analytical run to analyze all or those that are present. Mass spectrometric characteristics pertinent to quantitative analysis will also be emphasized.

Much attention has been directed to gas chromatography-mass spectrometry (GC-MS) analysis of morphine and codeine. Since other opiates, such as hydrocodone, hydromorphone, oxycodone, and oxymorphone, may interfere with the analysis of morphine and codeine and the analysis of these compounds themselves are also important issues, two double-derivatization approaches utilizing hydroxylamine (HA) and methoxyamine (MA) to first form oxime products with keto-opiates have been reported. The first approach adapted HA, followed by the derivatization with trimethylsilyl (TMS), while the second approach utilized MA, followed by pentafluoropropionyl (PrA) or TMS derivatizations. A review of the literature indicated that studies involving HA were limited, while the MA/PrA studies were (a) unable to chromatographically separate codeine and oxycodone; (b) unable to derivatize the hydroxyl group of oxycodone; and (c) did not include noroxycodone. On the other hand, the MA/TMS studies (a) did not include oxymorphone and noroxycodone; and (b) intensity cross-contributions between the ions designated for the analytes and their deuterated internal standards are generally very significant; thus, limiting the quantitation capability of this approach.

This study included a comprehensive list of compounds: codeine, morphine, 6-acetylmorphine, hydromorphone, oxymorphone, hydrocodone, oxycodone, and noroxycodone. Three-step derivatization approaches involving various combinations of derivatization groups were explored. Combination of MA/acyl/TMS was found to be most favorable. Merits of this approach include: (a) all functional groups in all analytes were derivatized; (b) the resulting products were chromatographically well resolved (Figure 1); and (c) intensity cross-contribution between the ions designated for these analytes and their respective deuterated internal standards were also found favorable (Table 1). Parallel approaches utilizing HA produced inferior results.



Ret. time	Derivatization product	Ret. time	Derivatization product
5.24	Codeine-TMS	5.40	Hydrocodone-MA
5.78	Oxycodone-MA-TMS	5.89	Codeine-PrA
6.16	Oxycodone-MA-PrA	6.18	Morphine-PrA-TMS
6.30	Hydromorphone-MA-PrA	6.60	6-Acetylmorphine-PrA
6.81	Oxymorphone-MA-PrA-TMS	7.21	Morphine-2PrA
8.62	Noroxycodone-MA-PrA-TMS		

Figure 1. Ion chromatogram of multiply derivatized opiate mixture.

Table 1. Cross-contribution (CC) data of ions (*m/z*) with potential for designating the analyte and the adapted internal standard (IS)

Derivatization product	Ion designating the analyte/IS and CC (in parentheses)
Codeine-TMS	313 (4.53) / 316 (4.83), 343 (4.66) / 349 (0.10), 371 (0.27) / 377 (0.11)
Hydrocodone-MA	297 (0.084) / 303 (0.00), 298 (0.00) / 304(0.00), 328 (0.00) / 334 (0.00), 329 (0.00) / 335 (0.00)
Oxycodone-MA-TMS	326 (4.25) / 332 (3.23), 401 (0.00) / 407 (0.03), 416 (0.00) / 422 (0.07), 417 (0.00) / 423 (0.06)
Codeine-PrA	282 (0.31) / 288 (0.01), 298 (0.35) / 304 (0.04), 355 (0.33) / 361 (0.00), 356 (0.44) / 362 (0.00)
Oxycodone-MA-PrA	230 (0.00) / 236 (0.00), 295 (0.00) / 301 (0.00), 343 (0.00) / 349 (0.00), 400 (0.00) / 407 (0.00)
Morphine-PrA-TMS	357 (1.92) / 360 (1.12), 413 (0.32), 416 (1.43)

Derivatization product	Ion designating the analyte/IS and CC (in parentheses)
Hydromorphone-MA-PrA	283 (0.56) / 289 (0.036), 314 (0.50) / 320 (0.004), 315 (2.32) / 321 (0.21), 339 (0.00) / 345 (0.31), 370 (0.12) / 376 (0.009)
6-Acetylmorphine-PrA	215 (4.19) / 218 (1.83), 268 (1.97) / 271 (1.55), 383 (0.17) / 389 (0.00), 384 (0.21) / 390 (0.00)
Oxymorphone-MA-PrA-TMS	215 (4.96) / 218 (1.87), 402 (0.94) / 405 (1.40), 403 (1.88) / 406 (0.70), 443 (0.085) / 446 (1.52), 458 (0.059) / 461 (1.64)
Morphine-2PrA	268 (2.02) / 274 (0.04), 324 (0.16) / 330 (0.04), 341 (0.24) / 347 (0.00), 342 (0.63) / 348 (0.00), 397 (0.15) / 403 (0.02)
Noroxycodone-MA-PrA-TMS	427 (0.00) / 430 (0.00), 458 (0.00) / 461 (0.00)

Opiate, Derivatization, Internal Standard

K15 Analysis of Amphetamines in Postmortem Matrices Using In-Matrix Alkylchloroformate Derivatization and Automated HS-SPME Followed by GC-MS

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The goal of this presentation was to evaluate the application Solid Phase Microextraction (SPME) and analysis for amphetamines in various postmortem matrices.

This presentation will impact the forensic community and/or humanity by demonstrating an automated extraction method for postmortem samples.

The extraction process of the sample for forensic toxicological analysis is necessary for the testing of analytes, such as drugs or poisons. A recent method of sample extraction known as SPME offers a number of advantages compared to previous extraction methods. SPME combines several phases of sample preparation (such as extraction, concentration, derivatization, and transfer to the analytical instrument) in one step. This can lead to a great reduction in sample preparation time, as well as solvent usage necessary to complete the sample preparation. Additionally, the application of autosampler provides a fully automated SPME sample preparation process.

Limited knowledge is available in the literature in terms of the application of SPME techniques for postmortem tissue samples (i.e., brain, liver, and kidney) and body fluids other than blood and urine (i.e., vitreous humor, bile, stomach, intestinal fluids, and saliva). These tissue and body fluids are important sources for forensic toxicological analysis; therefore, the development of SPME method for tissue samples is needed. In this study, the application of HS-SPME method for forensic toxicological analysis of postmortem samples was examined.

This study performed the analyses of amphetamines by automated HS-SPME-GC-MS. Amphetamines as a class are commonly abused, and therefore are frequently tested in postmortem toxicology laboratories. The researchers have adapted the in-matrix alkylchloroformates derivatization method for amphetamine analysis in the blood matrix. Additionally, the application of this method for automated HS-SPME was examined.

The sensitivity of this method as expressed limits of detection was 10 ng/mL for the amphetamines tested in the blood matrix. The precision study indicated the results obtained using this method were reproducible for amphetamine, methamphetamine, and methylenedioxy-methamphetamine. Additionally, the accuracy of the method was within 20% variation of the spiked concentration. In contrast, the precision study showed the results obtained using this method was were not reproducible for methylenedioxyamphetamine. Moreover, the accuracy of the method was more than 20% variation of the spiked concentration for methylenedioxyamphetamine in the blood matrix. The underlying causes of these effects need to be investigated in a future study.

The matrix effect study demonstrated that the biological fluid samples tested yielded higher signal responses than those of solid tissue samples. In addition to blood, the study indicated that stomach contents, urine, and vitreous humor were applicable for the SPME method. The matrix effects of the tissue matrices tested interfered with the SPME extraction of amphetamines tested; thus, they were unlikely applicable for the HS-SPME method used in this study.

Amphetamines, Alkylchloroformate, Solid Phase Microextraction

K16 Isotopic Dilution in the Analysis of Cocaine, Cocaethylene, and Benzoylecgonine in Whole Blood: Comparison of the "Traditional" to the "Direct" Application of Deuterated Drug Analogs

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After attending this presentation, attendees will obtain a better understanding of mass spectral analyses incorporating isotopically labeled drug analogs as internal standards. A review of the traditional use of isotopically labeled drug analogs and, more importantly, their use in an expanded analytical role is presented. The attendee will obtain an understanding of Response Factors and Relative Response Factors in quantitative mass spectrometric analyses.

This presentation will impact the forensic community and/or humanity by demonstrating that costs can be greatly reduced, without sacrificing analytical reliability, by lowering the number of GC/MS injections necessary, ultimately resulting in decreased instrument time. Costs associated with analyst's time are also greatly reduced because only four extracts are necessary: (1) a single calibration standard to verify the Relative Response Factor (RRF) and to establish the necessary qualitative criteria (retention times, ion ratios, etc), (2) a positive control, (3) a negative control, and (4) the specimen. This direct analytical approach greatly simplifies analysis of blood samples containing multiple analytes because additional isotopically labeled drug analytes can be added at the beginning of the extraction sequence.

Traditionally, laboratories use isotopically labeled drug analogs as internal standards for quantitating drugs in biological specimens. Calibration curves are constructed by plotting the response ratios, i.e., area abundances of drug-D_o/ area abundances of drug-D_n versus the concentrations of the drug–D_o. For purposes of this study D_o represents the drug analyte, and D_n refers to the tri-deuterated, isotopically labeled, drug analog.

In the "direct" application of deuterated drug analogs, the concentration of D_o in the specimen is determined directly from the amount of D_n used in the analysis by applying the following relationship:
ng/mL D_o = (Area abundance D_o/ Area abundance D_n x nmol D_n x 1 nmol D_o/1 nmol D_n x MW D_o) / mL specimen.

To validate this approach, the Relative Response Factor (RRF) for D_o relative to D_n over the range of expected results is established. The RRF is determined by considering the individual Response Factors (RF) for D_o and for D_n, where RF is defined as the magnitude of some measurable parameter divided by the amount giving rise to that measurement, or RF-D_o = area abundance D_o / nmole-D_o, and RF-D_n = area abundance D_n / nmole-D_n. The RRF (RF-D_o / RF-Dn) is determined for each point in the range. Theoretically, an RRF of 1.0 over a given range indicates that the mass spectrometer response to D_o is identical to the response to D_n for that range.

Mathematically, RRF = RF-D_o / RF-D_n ; or RRF = Area D_o / nmole D_o or Area D_o / Area D_n Area D_n / nmole D_n nmole D_o / nmole D_n which, on rearrangement results in (Area D_o / Area D_n) = RRF (nmole D_o / nmole D_n) and represents a linear relationship in the (y = mx + b) format. By plotting (Area D_o / Area D_n) as y; (nmole D_o / nmole D_n) as x; and setting b to 0, the slope (RRF) is obtained.

This study demonstrated that the RRFs for cocaine, cocaethylene and benzoylecgonine were 1.01, 0.99, and 0.99 respectively over the range of 20 – 2000 ng D_o versus 100 ng D_n with R^2 values greater than 0.99 for each, which indicated that the mass spectrometer response to D_o was the same as the response to D_n over that range and allowed for the D_o concentration in a whole blood specimen to be determined directly from the amount of D_n used in the analysis.

Drug Analyte (Drug-D0), Deuterated Drug Analog (Drug-Dn), RRF (Relative Response Factor)

K17 Simultaneous Extraction of Pesticides From Human Adipose Tissues and GC/MS Detection

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After attending this presentation, attendees will understand the value of retaining alternative tissues for postmortem toxicological analyses.

This presentation will impact the forensic community and/or humanity by demonstrating the utility of alternative postmortem tissue analysis in determining defensible cause of death. The objective of this presentation is to relate experiences regarding use of adipose tissue, as a supplement to blood and other organs, for the postmortem identification of pesticides.

A modified method is presented[1] for the efficient extraction of pesticides from human adipose tissue. The procedure combines purification, extraction on Extrelut column and GC/MS analysis.

5 g adipose tissue pesticide free homogenate was spiked with 2.8 mcg/g of a mix of pesticides (73 ng/mL Dichlorvos, Fludioxonil, Methiocarb, Methomil, Chlorpyrifos, Thiamethoxam, Tebufenpyrad, Tebuconazol, Quinoxyfen, Pyrimethanil, Penconazol). The mixture was vortexed for 15 seconds and 10 g of anhydrous sodium sulphate and 0.5 g of tartaric acid added.

The homogenate was extracted three times with petroleum ether, followed by evaporation of the ether layer at room temperature under N_2. The residue was reconstituted in 20 mL of petroleum ether, filtered, and extracted with 5 mL of acetonitrile saturated with petroleum ether. 100 mL of a 5% NaCl aqueous solution was added to the acetonitirile/ether phase and extracted with an additional 10 mL of petroleum ether. The extract was again evaporated at room temperature under N_2.

5.5 g of florisil was activated (120°C, 30 min), and placed in an Extrelut column (Merck). The extract was reconstituted with 2.5 mL of petroleum ether and added to the column. The column was then eluted with 50:50 ethyl ether/petroleum ether and the eluate evaporated at room temperature under N_2. The residue was then reconstituted and injected into a GC/MS-EI operating in full scan mode.

This extraction procedure for pesticides in human adipose tissue was evaluated on the basis of accuracy, reproducibility, and chromatographic profile. The method is simple and rapid and produces relatively clean extracts, suitable for gas chromatography/mass spectrometry full scan EI analysis. The acidic purification/solid-phase extraction provided best compromise between recovery and chromatographic profile.

References:

[1] Locani O.L.; Perkins de Piacentino A.M.; Ginesín L.M., Mangas L., Matrices Alternativas: Tejido Adiposo una Matriz de Eleccion, Cuadernos de Medicina Forense, 2005: 2 (3): 29-40

Adipose Tissue, Alternative Tissue, Pesticide

K18 Detection of Biomarkers of Explosives

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After attending this presentation, attendees will become familiar with an approach to detect biomarkers of explosives using screening and low level detection techniques.

This presentation will impact the forensic community and/or humanity by providing novel screening methods that will extend the detection time and concentration ranges of metabolized explosives.

Detecting explosive biomarkers in human biological fluids can be useful in identifying individuals who have either handled or been exposed to explosive compounds. In order to fully implement such detection, methods ranging from screening to trace level detection of metabolites are needed. This presentation will discuss two such techniques applied to volatile biomarkers of explosives.

Explosive compounds may enter the body via inhalation or skin absorption and undergo metabolism. Once the explosive compounds are metabolized, the metabolites may be present in blood and urine. The volatility of these explosives and their metabolites may provide alternative means for detecting them in biological fluids. Finding unique metabolites also referred to as biomarkers, in biological samples will give forensic toxicologists a valuable investigative tool that can assist in identifying people who have handled explosives. The concentration of these biomarkers in the body may be too low to detect using standard analytical techniques. For trace level metabolites, a preconcentration technique such as purge and trap gas chromatography/mass spectrometry (PT GC/MS) is ideal for detecting volatile metabolites in biological matrices. Another method, ion mobility spectrometry (IMS), is a rapid and sensitive screening method with low detection limits. IMS is widely used for the detection of trace explosive compounds; however, minimal research has been reported using direct headspace samples of explosives with IMS. The present work employed using PT GC/MS and IMS as screening methods for the detection of volatile explosives metabolites in headspace. A loop and a trap method for the PT GC/MS were used for this analysis. The samples were incubated at body

temperature, thirty-seven degrees, for twenty minutes prior to purging. The ability to detect explosives metabolites in biological matrices is time limited because the body metabolizes substances at various rates. Having the capability to preconcentrate using PT GC/MS gives a wider range of time and concentrations to analyze trace metabolites in real biological samples. The advantage of detecting low concentrations with IMS will assist in rapid screening of explosives metabolites in headspace. In the future, this research may aid in the development in a method which would detect explosives metabolites in breath.

Methods for detecting explosives metabolites in headspace of biological matrices can be useful to the investigation of bombers and bomb-makers. This study primarily focuses on TNT and its metabolites, 2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene and dinitrotoluene. Other explosives will also be discussed in this presentation. This information will present headspace data obtained in urine and blood by PT GC/MS and IMS.

IMS, PT GC/MS, Explosives Metabolites

K19 Extraction and Analysis of Warfarin From Whole Blood Using a Long Chain SPE Sorbent

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The goal of this presentation is to present information on a solid phase extraction method that will improve on existing procedures for the analysis of warfarin in postmortem blood samples.

This presentation will impact the forensic community and/or humanity by improving the analysis of this drug in post mortem samples by utilizing a more efficient extraction system i.e., a long chain SPE sorbent in conjunction with both liquid and gas chromatographic systems.

Warfarin (Coumadin) is a popular pharmaceutical used as a blood-thinning agent. In therapeutic use, blood levels range from 1000 ng to 3100 ng per mL has been reported.[1] Several methods have been used for the analysis of this drug using liquid-liquid extraction.[2,3] This project was developed in order to study this drug at low levels in post mortem samples using a novel (C_{30}) solid phase sorbent.

In this method, Warfarin and the internal standard (p-chlorowarfarin (100 ng)) were spiked into whole blood samples (1mL) over a concentration range 0 through to 200 ng per mL. The samples were treated with an aqueous phosphate buffer (9 mL) and the drugs extracted onto a C_{30} SPE columns (200 mg). The columns were washed with the phosphate buffer and hexane (1x 3 mL each) and eluted with 14% methanol acid in ethyl acetate (2x 3mL). The eluents were collected and evaporated for further chromatographic analysis. Using GC-MS, the samples were derivatized prior to analysis using BSTFA, for analysis with LC-PDA the samples were reconstituted in DI water.

GC-MS separation was carried out using an Agilent Technologies 6890 GC coupled to a 5975 MSD for SIM analysis. HPLC analysis was carried isocratically out using both PDA and Fluorescence detection.

From this method LOQ's of 25 ng per mL of sample is easily achievable by either chromatographic system. By using GC-MS (SIM) in EI mode, 10 ng per mL of sample can be detected.

Examples of chromatograms and calibration curves are presented to show the simplicity and efficiency of this methodology.

References:

[1] C.Winek *et al*, Forensic Sci. Int'l 122 (2001) 107-123

[2] Locatelli *et al*, J.Chrom.B., 818 (2005) 191-8

[3] Naidong *et al*., J.Pharm.Biomed. Anal. 25 (2001) 219-29

Warfarin, SPE, Toxicology

K20 Stability of Exogenous GHB in Antemortem Blood and Urine Under Various Temperature and Storage Conditions

Albert A. Elian, MS, Massachusetts State Police Crime Laboratory, 59 Horse Pond Road, Sudbury, MA 01776*

After attending this presentation, attendees will learn how storage conditions will effect GHB concentration in blood and urine.

This presentation will impact the forensic community and/or humanity by demonstrating the effect of long storage on GHB levels in blood and urine.

The stability of exogenous GHB in three blood and three urine samples under a variety of storage conditions; room temperature, 4°C and -20°C, was evaluated over a period of six months. GHB concentration increased the most at room temperature, with almost no change at the lower temperature.

Gamma-hydroxybutyric acid (GHB) is an endogenous substance found in the body. This central nervous system depressant, which was first synthesized in the 1960s, has been used for induction of anesthesia, treatment of narcolepsy, and for alcohol and opiates withdrawal. Recently, GHB has been used illicitly by bodybuilders to increase the release of growth hormone, ravers attendees for its euphoric, sedation and muscle relaxation after ecstasy use, and victims of drug-facilitated sexual assault (DFSA) [8-10].

Due to the increased demands on forensic toxicologists to analyze GHB in cases such as DFSA and operating motor vehicles under the influence, there are often variable time intervals between collection of the specimen and analysis. A literature review has revealed no stability study on antemortem blood or urine exogenous GHB levels. However, one study reported the effect of storage on endogenous GHB antemortem urine levels, and another study investigated the effect of storage conditions on GHB-free and spiked urine antemortem concentration.

Quantitation of GHB was achieved by liquid-liquid extraction, followed by concentration of the extracts and derivatization with BSTFA. Analysis was performed on an Agilent 6890 gas chromatograph interfaced with an Agilent 5975 mass selective detector. A 12m x 0.25mm (internal diameter), 0.25mm (film thickness), HP-1MS column (100% polydimethylsiloxane) was used with helium as the carrier gas at a flow rate of 2.0 mL/min. An Agilent 7683 automatic sampler was used for injection into the gas chromatograph. The splitless injection mode was used with the valve closed for 0.25 min, and 2ml samples being injected. The operating conditions for the analyses were injection port, 280°C; the detector, 300°C; initial oven temperature, 60°C for 2 min increased at 30°C/min to 300°C, holding for 1 min. The mass spectrometer was operated in the SIM mode. The ions selected for monitoring were chosen from full scan mass spectral analyses of the analytes that gave minimum interference. The following ions were monitored: GHB: m/z 233,234,235 and GHB-d_6: m/z 239,240,241.

Three actual blood (20, 50, and 75 mg/L) were submitted to the laboratory in test tubes containing sodium fluoride. Three actual urine samples (33, 108, and 220 mg/L) were submitted in plastic jars with no preservative added. The samples were chosen, from casework, to cover a wide range of concentrations. The specimens were analyzed at the time of arrival in the laboratory and then divided into three sets as described above.

For the blood stored at -20°C there was an increase in GHB concentration of 1-12%, at 4°C 3.4-16%, and 20°C 9.6-28% (Fig. 1-3). For the urine stored at -20°C there was an increase in GHB concentration of 1-15%, at 4°C 1-27%, and at 20°C 3.6-44% (Fig. 4-6), with the highest increase in GHB concentration in the lower concentrations (Fig. 1 and 4). This could be attributed to the fact that a small increase in the GHB level would be enough to significantly change to the measured level.

Storage, Gamma-Hydroxybutyric Acid (GHB), Exogenous

K21 Evaluation of the Immunalysis® Fentanyl ELISA Assay for Use in Screening Postmortem Blood

Denice M. Teem, BS, Daniel S. Isenschmid, PhD, Bradford R. Hepler, PhD, and Carl J. Schmidt, MD, Wayne County Medical Examiner's Office, 1300 East Warren Avenue, Detroit, MI 48207*

After attending this presentation, attendees will be aware of a rapid ELISA screening test for fentanyl in postmortem blood that is sensitive, specific, and efficient.

This presentation will impact the forensic community and/or humanity by providing the toxicologist with data to aid in the selection of a reliable screening assay for fentanyl in postmortem blood.

Since 1999, the Wayne County Medical Examiner's Office (WCMEO) has routinely looked for fentanyl in its GC/MS screen (LOD, 5 ng/mL). The incidence of cases in which fentanyl was identified was 6 (1999), 3 (2000), 7 (2001), 12 (2002), 20 (2003) and 29 (2004). These gradual increases were largely due to increased use and abuse of fentanyl patches and lollipops. Fentanyl confirmation and quantitation had been performed by a reference laboratory until May 2005 when the WCMEO developed a GC/MS-SIM confirmation method (LOD, 1ng/mL). Due to an outbreak of fentanyl-laced cocaine and heroin in the last 3 months of 2005, the incidence of fentanyl increased to 63 in 2005 and 132 for the first six months of 2006. Due to the surge in fentanyl-related cases, it was necessary to add an immunoassay to allow the laboratory to rapidly identify potential fentanyl-related cases and to expand its fentanyl screening protocol to all autopsied cases.

The Immunalysis® ELISA fentanyl assay was evaluated for use in screening postmortem blood using Tecan® equipment. Pipetting was performed on Miniprep 75 using a 1:10 specimen dilution without any sample pretreatment. Plates were washed with a Columbus II plate washer and read using a Spectra II plate reader.

Pooled postmortem negative blood (as determined by ELISA and GC/MS) was used as a negative calibrator. A cut-off calibrator (2 ng/mL) and controls (1 ng/mL and 4 ng/mL) were prepared in-house by fortifying blood from the negative blood pool.

The within-run precision and linearity around the cutoff of the fentanyl assay was determined by assaying negative, 1, 2 and 4 ng/mL calibrators and controls (N=16) interspersed throughout a single plate. The assay demonstrated good precision and excellent separation as summarized in Table 1.

Table 1

Concentration	%CV	Average A/Ao	SD
Negative	5.27	97.81	5.15
1 ng/mL (low control)	6.73	47.04	3.16
2 ng/mL (cutoff)	7.64	26.42	2.02
4 ng/mL (high control)	11.72	14.84	1.74

Between-run precision was assessed by using the A/Ao obtained for the low and high controls that were assayed three and four times, respectively, in each ELISA batch. For the high control the CV was 13.2% (N=24). The low control had a CV of 13.3% (N=18). These CV's appeared to reflect the variation in ELISA assays and were not a result of pipetting imprecision as the Miniprep 75 demonstrated a CV of only 0.11% for the pipetting of the sample and diluent (N=64). ELISA assays typically demonstrated higher CV's than traditional immunoassays, however by calibrating using the mean of duplicate negative and cutoff calibrators, and due to the excellent separation around the cutoff calibrator, there were no failed controls on any batch ran.

Sensitivity, specificity, and efficiency were evaluated comparing the ELISA and GC/MS results with 314 blood specimens. These included a series of known positive samples and a series of sequential blood specimens analyzed in sequence as per routine casework. There were 225 true negatives and 88 true positives. The single false positive by ELISA was readily explained by the 1.9 ng/mL GC/MS concentration that was just below the cutoff. There were no false negatives. This resulted in excellent sensitivity (98.9%), specificity (99.6%), and efficiency (99.7%).

Fentanyl, ELISA, Method Evaluation

K22 Capillary Electrophoresis/Electro-Spray Ionization/Time-of-Flight Mass Spectrometry of Low Dose Benzodiazepines

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The goal of this presentation is to determine the usefulness of capillary electrophoresis electro-spray ionization time-of-flight mass spectrometry (CE-ESI-TOF-MS) for the detection of low dose benzodiazepines.

This presentation will impact the forensic community and/or humanity by providing a new approach to accurately and consistently determine the presence of benzodiazepines at low concentrations. This technique can then be applied to cases where drug facilitated sexual assault is suspected.

Besides being used for therapeutic purposes benzodiazepines are commonly abused at parties, night clubs, and raves because of their ability to cause euphoria and a drunk-like high. Common low dose benzodiazepines include alprazolam, clonazepam, lorazepam, and triazolam. When benzodiazepines are taken in the presence of alcohol, the user experiences heightened effects as well as amnesia and unconsciousness. In such instances the drug is often used as a tool in drug facilitated sexual assault and can be present in such low concentration that it is often difficult to detect. In cases of sexual assault, the type, concentration, and number of drugs taken can aid in the investigation. However, there are few methods capable of fully determining this information.

Recently, capillary electrophoresis has been proposed as an alternative to GC/MS for toxicological analysis, especially when coupled to electrospray mass spectrometry (ESI-MS). CE has several advantages which make it an ideal method to couple with ESI-MS. It has high efficiency, minimal sample requirements, and short analysis time. MS is an analytical technique that can reveal specific, characteristic, and structurally related information about a compound. The analytes must be present in the vapor phase, and this condition is obtained via electrospray analysis.

The present work was performed on an Agilent CE-ESI-TOF-MS which utilizes a sheath flow interface to spray a minimal and regulated amount of the eluted CE sample into the TOF. The TOF provides several advantages when used in combination with CE. The system is very fast compared to trap systems or quadrupoles and has a 3ppm or less mass resolution, allowing extremely accurate empirical formula determination based on high resolution mass determination. The system identifies drugs based on four operational parameters: absolute mass, prediction of related absolute isotopic mass abundances, in-source collisional dissociation, and electrophoretic mobility. The high separation efficiency of CE combined with the high sensitivity and information content of MS makes this instrument a powerful tool for screening and confirmation of drugs. These characteristics make ESI-TOF one of the more suitable mass spectrometric detection methods for CE.

Selected benzodiazepines were analyzed using this system, and extracted ion analysis was performed with high selectivity by using the exact masses of the protonated molecular ions. This capability greatly reduced background noise and improved detection. The effect of buffer

pH, concentration, and spray parameters were also examined. The capillary column used had a 75 micron internal diameter and a length of 95mm. The polyimide coating was removed from the end of the capillary and a blunt tip was cut in the fused silica with a sapphire scribe. This was crucial when using the CE-MS to obtain a perfect and precise spray while eliminating adsorption of the sample on the outside of the capillary. The running buffer used was 20mM formic acid at a pH of 2.7. At this low pH the drugs were cationic, and, although a low electro-osmotic flow (EOF) resulted, electrophoretic mobility and induced flow resulting from the sheath liquid during analysis enabled overall run times of under 8 minutes. The results demonstrate CE-ESI-TOF to be a rapid and highly specific detection method for benzodiazepines.

Capillary Electrophoresis, Time-of-Flight Mass Spectrometry, Benzodiazepines

K23 Cases of Insulin Poisoning

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After attending this presentation, attendees will be briefed on cases of poisoning due to exogenous insulin administration and the measurement of insulin in forensic specimens.

This presentation will impact the forensic community and/or humanity by demonstrating the identification of insulin and C-peptide in forensic specimens.

Hypoglycemia caused by deliberate or inadvertent administration of insulin is a potentially lethal disorder. Self-induced hypoglycemia by clandestine use of insulin was reported in 1982 by a nurse seemingly with the sole purpose of attracting attention and sympathy. Since that report, similar cases have been described in diabetics as well as non-diabetics. Although it is clear that insulin induced hypoglycemia can cause death, in most cases of death occurring within 24 hours of proven hypoglycemia, classic autopsy procedures reveal no unique pathophysiologic abnormalities. After B-cell stimulation by carbohydrate intake, insulin and C-peptide are secreted in 1:1 molar ratio. A large portion of insulin is cleared by the liver, while the C-peptide which is primarily cleared by the kidney has a lower metabolic clearance. In normal physiology, the molar ratio of insulin to C-peptide in endogenous secretion should be less than one. Hypoglycemia caused by exogenous insulin is associated with high serum levels of insulin and low serum levels of C-peptide. Thus, the ratio of insulin to C-peptide should be greater than one. The following four cases were recorded as possible overdose of insulin.

Case Study 1: A 53-year-old white female was found unresponsive in a motel room with numerous syringes lying around the body along with Humulin. An alleged suicide note was recovered by the police. Medical records indicated that the deceased was an insulin dependant diabetic with cardiac history. Police reports also indicated that the decedent's girl friend reported that the decedent was going to commit suicide with an overdose of insulin if she broke off their relationship.

Case Study 2: A 20-year-old black female living with her male companion made statements regarding suicide on several occasions. The male companion who was a diabetic reportedly moved out with his supply of insulin and syringes and did not leave any behind. He called the police when he was unable to contact her, fearing she had committed suicide. Police found a secured house. There was no evidence of injury, but an empty insulin bottle was found on the scene. Autopsy revealed a gravid uterus with a normally developed fetus.

Case Study 3: A 34-year-old white female was discovered deceased in her motor vehicle. Inside the vehicle were found insulin packages and syringes, including some empty insulin bottles. A hand written note to her psychiatrist was also found stating "forgive me Dr." According to the spouse, the decedent made prior suicide attempts; one of them with overdose of insulin.

Case Study 4: A 38-year-old white male with morbid obesity was found deceased in his residence. The decedent had a history of diabetes and had accidentally sustained a gun shot wound several years ago. The projectile was still believed to be in his back. The decedent was depressed and told his relatives that if he continued to not feel well he was going to kill himself with an over dose of insulin.

Insulin and C-peptide results are shown in the table below.

Case Number	Insulin uIU/mL	C-Peptide ng/L	Insulin/C-Peptide Ratio	Diagnosis
Case 1	4.3	0.3	0.3	Cardiovascular disease
Case 2	88.5	0.1	18.7	Suicide Insulin Overdose
Case 3	524	0.1	110.6	Insulin Overdose
Case 4	5.0	0.1	1.1	Cardiomegaly

Specimens were analyzed for Insulin and C-Peptide by RIA, using commercially available reagent kits from Diagnostic Systems Laboratories (Webster, TX). Since post-mortem specimens were highly hemolyzed, the protocols were modified to include standard addition recovery and serial dilution for each specimen analyzed. Further validations included comparison between duplicate results of insulin and C-peptide on the same specimens analyzed by the authors' laboratory and Mayo Clinic Laboratories; correlations by Pearsons r were 0.82 and 0.99, respectively. The ratio cutoff was validated from clinical specimens. For example, a specimen was obtained from a patient suspected of insulinoma and analyzed for insulin and c-peptide; insulin concentration was 18.1 uIU/mL, and C-peptide was 19.7 ng/mL, providing a molar ratio of 0.02. Eleven specimens obtained from diabetic patients who are on insulin therapy were also analyzed for insulin and C-peptide. All of them had C-peptide levels approaching the assay's detection limit, and insulin:C-peptide ratios were 1 or less. Typical units for insulin and C-peptide levels are as number of moles. The ratio was calculated with the formula Insulin:C-peptide Molar Ratio = (Insulin uIU/mL ÷ C-peptide ng/mL)(0.0211). The ratios obtained were consistent with the literature. Moreover, the ratios from appropriate medical use of insulin can be distinguished from insulin overdose. These results confirm the use of "Insulin/ C-peptide" ratio in suspected insulin overdose, using commercial RIAs.

Insulin Poisoning, Syringes, Suicide

K24 Effect of Torso Dart Position and Cocaine Intoxication on Taser® Induction of Ventricular Fibrillation

Patrick J. Tchou, MD, Dhanunjaya Lakkireddy, MD, and Donald Wallick, PhD, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195*

After attending this presentation, attendees will learn about torso dart positions as they relate to the propensity to induce ventricular fibrillation (VF) by neuromuscular stun guns as well as the effects of cocaine on VF induction thresholds by stun guns.

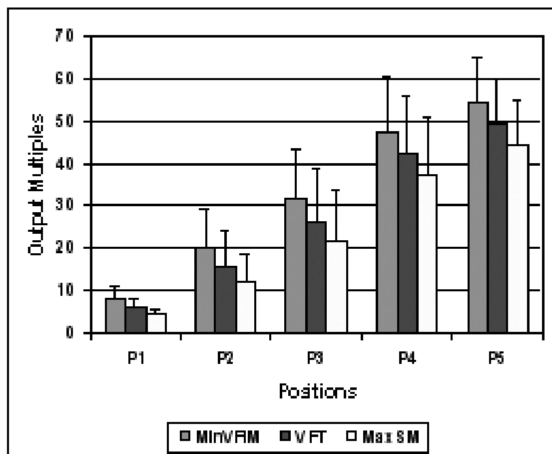
This presentation will impact the forensic community and/or humanity by assessing the likelihood that stun guns contribute to induction of ventricular fibrillation in such subjects.

Neuromuscular stun guns are increasingly used by law enforcement to restrain uncooperative and combative subjects being taken into custody. Multiple deaths have been reportedly associated with the use of stun guns. This study aimed to assess the threshold for ventricular

fibrillation (VF) induction using the Taser® X26 waveform with darts positioned at various common torso locations in an anesthetized pig model. The effect of cocaine on the VF induction thresholds was also investigated.

Thirteen pigs (34±7 kg) were utilized in the initial study to assess dart locations on VF induction thresholds. Dart positions on the torso were as follows: 1) Sternal notch (SN)to cardiac apex 2) SN to supraumbilical area 3) SN to infraumbilical area 4) side to side on the chest 5) superior to inferior region of the dorsum. Increased outputs of the Taser® X26 waveform was delivered by increasing the capacitance of the stored charge in a custom built device. VF thresholds were determined by a stepwise increasing output until VF was induced by a multiple of the baseline capacitance. VF thresholds were also measured in five pigs after infusion of cocaine at 8 mg/kg over 30 minutes.

Results are shown in the following figure.



MinVFIM = minimum capacitance multiple inducing VF
Max SM = maximum multiple not inducing VF on 3 consecutive applications
VFT (VF threshold) = average of MinVFIM and MaxSM
P1-P5 = Positions 1 through 5

Cocaine consistently increased MinVFIM, MaxSM and VFT at all positions by 50% to 200%. Intracardiac electrograms demonstrated that VF induction was related to rapid ventricular capture by the Taser® pulses.

Conclusions: NMI output equivalent to standard Taser® X26 did not induce VF in any pig. Position of the darts significantly affects propensity for induction of VF being most sensitive in the precordial position. Cocaine increased VFT probably through its sodium channel blocking properties.

Neuromuscular Stun Guns, Ventricular Fibrillation, Cocaine

K25 Enforcement of DUID Laws in European Nations — Sweden's Zero Limit Blood Law and Case Examples

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After attending this presentation, attendees will obtain an overview of the way drug-impaired driving is dealt with in European nations. The main focus will be on new legislation introduced in Sweden where zero-concentration limits in blood are enforced for both licit and illicit drugs, if these are classified as controlled substances. The forensic community in North America will learn the effectiveness of so-called zero tolerance legislation as a way to simplify the prosecution of DUID offenders and hopefully improve traffic safety.

This presentation will impact the forensic community and/or humanity by demonstrating the effectiveness of so-called zero-tolerance laws for driving under the influence of drugs (DUID) as a new countermeasure to improve traffic safety. Since the introduction of a zero-limit DUID law in Sweden the number of people apprehended by the police for this traffic crime has increased 10-fold. In the vast majority (85%) of such cases one or more banned substances are detected in blood samples and the prosecution and conviction of DUID has become much more streamlined.

Legislation pertaining to driving under the influence of drugs (DUID) has evolved from the pre-existing alcohol-impaired driving laws, which have a long history. The statutory limits of blood-alcohol concentration (BAC) for driving have decreased successively from 0.15 g/100 mL to 0.1 g/100 mL and are presently set at 0.08 g/100 mL in UK, USA, and Canada. This contrasts with the corresponding threshold BAC limits in most European nations of 0.05 g/100 mL and 0.02 g/100 mL in Norway and Sweden. The notion that DUI laws are science-based is clearly a myth as evidenced by this wide range of punishable concentrations - reflecting, of course, politics, rather than traffic safety research. The success of concentration *per se* laws as evidence of alcohol-impaired driving has prompted similar discussions for DUID legislation. Such a legal framework shifts the focus of the prosecution case away from evidence of driver impairment towards the concentration of a banned substance determined in a specimen of blood obtained from the suspect. The actual driving, the behavior of the suspect when questioned, and performance of skilled tasks, become supporting evidence in the prosecution case.

Studies aimed at finding a quantitative relationship between the concentrations of illicit drugs in blood and degree of diminished performance and impairment of the individual are few and results are often equivocal. There are many ethical constraints about the design of such studies including selection of subjects, the dose of drug administered and the suitability of the performance tasks. This stems, at least in part, from the complex nature of drug-related impairment and the time-lag between the blood-drug concentration and the onset of drug-related effects as well as after-effects or rebound phenomena and withdrawal. The situation is complicated still further by habituation to drugs, especially those with long half-lives, which tend to accumulate in blood after repetitive use and leads to the development of physiological tolerance. Moreover, many prescription drugs impair a person's ability to drive safely and some have pharmacologically active metabolites that exert their own effects on a person's performance and behavior. Effective DUID legislation cannot ignore the widespread use and abuse of medicinal drugs; anti-anxiety agents, sedatives, hypnotics and pain-killers and the associated performance decrement these cause.

The impetus to consider seriously a zero-limit blood law for drugs other than alcohol arose from media attention given to several high profile DUID cases. A female driver (30 y) was stopped by the police during a routine traffic control. A preliminary breath-alcohol test was negative but one of the police officers noticed that the woman's eyes were bloodshot and that pupils were dilated. This raised a suspicion of DUID and a blood sample was requested for toxicological analysis. Otherwise the suspect did not show any marked signs and symptoms of drug influence and she was not examined by a physician nor were field-sobriety tests performed. The toxicology report showed a high concentration of amphetamine (3.4 mg/L) as well as phenmetrazine (0.2 mg/L), both widely abused central stimulants in Sweden. The woman was eventually prosecuted for DUID based on the toxicology report and opinions from several expert witnesses about the effects of such high levels of amphetamine on a person's ability to drive safely. However, the woman was acquitted in both the lower court and the appeal court because of the lack of well-documented clinical evidence of impairment and the fact that a traffic violation had not been committed. According to the court there was no compelling evidence to prove the suspect was "under the influence" of a central stimulant and posed a danger to traffic safety.

Other examples of widely divergent results between clinical assessment of impairment and the toxicology findings helped to spark the debate about considering zero-concentration limits in blood for drugs other than alcohol. In one notable case a 34-year-old woman was found to be slightly under the influence of a stimulant or depressant drug according to a clinical examination by a forensic physician. The forensic toxicology report verified the presence of several scheduled drugs, both licit and illicit, in a blood sample; amphetamine (0.03 mg/L), phenmetrazine (0.1 mg/L), THC (0.001 mg/L), morphine (0.08 mg/L), codeine (0.02 mg/L) and very high concentrations of diazepam (3.6 mg/L) and its metabolite nordiazepam (7.8 mg/L). Analysis of urine showed high concentrations (>1 mg/L) of free-morphine, free-codeine and 6-acetyl morphine, which verifies the woman had also used heroin.

Poly-drug abuse is the norm in Sweden among DUID offenders. Since the introduction of the zero-concentration limit law for scheduled drugs in blood of drivers in 1999, the number of DUID cases submitted for toxicological analysis has increased more than 10-fold. In about 85% of these cases one or more banned substance is verified present in the blood specimen. The zero-limit law has stimulated police activity in apprehending DUID suspects, which has led to a substantial increase in the workload for the forensic toxicology laboratory. The analytical routines for dealing with DUID cases have been modified so that after an initial screening analysis of blood or urine by immunoassay methods (EMIT/CEDIA), only a single illicit substance is subjected to a quantitative analysis by substantive methods, such as GC-MS or LC-MS. The punishment for DUID in Sweden is the same regardless of how many illicit drugs are verified present in a blood specimen. The introduction of so-called zero-tolerance or LOQ laws furnishes a robust and pragmatic way to enforce DUID legislation, and this simplifies considerably the evidence required for a successful prosecution. However, such laws have done nothing to solve the problem of DUID because recidivism in these traffic delinquents exceeds 50% over a 4-year period.

Drugs, Driving, DUID

K26 "That's the Night That the Lights Went Out in Ventura" (A DUID Soma / Methamphetamine Case Study)

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After attending this presentation, the participant will receive insight into the events surrounding one of the most prominent "driving under the influence of drugs" cases adjudicated in Ventura County.

This presentation will impact the forensic community and/or humanity by illustrating the difficult challenges and decisions made by the toxicologists; investigators and prosecutors associated with drugged driving collision fatality cases. The detailed Case Study model format and its examination serve as a vehicle to share knowledge and promote discussion in this constantly evolving arena.

At 6:50 p.m. on August 12, 1998, the lights at the Telephone and Hill intersection near the Government Center in Ventura, California were inoperable. Each car was proceeding by treating it as a four-way stop, as is required by law. The first driver, a 28-year-old female senior Deputy, was preparing to turn left on Hill Street onto Telephone heading east. The second diver was in a pick-up truck on Telephone heading west traveling work at UPS. Traffic in the lanes was backed up as people were abiding by the inoperable signal. The second driver approached the intersection at a minimum speed of 56 miles per hour, made a late lane change into the right turn lane of Telephone road, and proceeded straight through the intersection crashing into the car being driven by the first driver. The impact was tremendous and the first driver probably died instantaneously. None of the law enforcement personnel noted any symptomologies. One even gave the opinion that the second driver was not under the influence of alcohol or drugs.

The second driver admitted only to having taken Claritan and Sudafed. She claimed Soma had been administered two days earlier. At 8:50 p.m. a urine sample was obtained and found to be positive for methamphetamine and Soma. At 9:10, blood was drawn from the second driver, which tested negative for the presence of alcohol but positive for methamphetamine, and Soma. The methamphetamine level was quantified to be at 0.12 milligrams per liter. The Soma level was quantified to be at 0.58 milligrams per liter.

The second driver had been a long-time methamphetamine user. In 1984, she possessed five pounds of methamphetamine to be delivered for sale and pled guilty in Federal Court on that case in 1988. She had tested positive for methamphetamine twice while on probation.

Six separate experts were consulted while preparing for trial in this case. Opinions varied on levels of impairment and the ability to prove a case beyond a reasonable doubt with these facts. The various opinions and their impact on the case will be discussed in the presentation of this case study.

Drugged Driving, Driving Impairment, Under the Influence

K27 Case Study: DUI With Multiple Prescription Drugs

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After attending this presentation, attendees will have been exposed to a case study involving drug impaired driving in which the toxicology results are bolstered by a video of the subject.

This presentation will impact the forensic community and/or humanity by demonstrating some of the impairing effects of Central Nervous System (CNS) depressant drugs and give the audience a better understanding of the physical effects of these drugs.

This case study involves a 39-year-old white male who is involved in a collision where he subsequently fled the scene. Eye witnesses were able to identify him to police and he was arrested at his home a short time later. The police in-car video shows a subject who is obviously under the influence of (CNS) depressants. After a breath alcohol reading of 0.00%, urine was obtained for toxicological analysis. This analysis revealed the presence of diazepam, nordiazepam, temazepam, alprazolam, hydrocodone, acetaminophen, dihydrocodeine, cyclobenzaprine, carisoprodol, and meprobamate.

With exception of acetaminophen, all of the drugs identified are prescription medications (or metabolites) that have CNS depressant actions. Diazepam is a benzodiazepine which is utilized clinically as an anxiolytic, anti-convulsant, sedative, and muscle relaxant. Nordiazepam and temazepam are active metabolites of diazepam which are also used clinically as sedative-hypnotics. Alprazolam is used primarily as an anxiolytic and to treat certain conditions such as agoraphobia and panic disorders. Hydrocodone is a semisynthetic derivative of codeine that is utilized as an analgesic and is often found in combination with acetaminophen. Dihydrocodeine is an active metabolite of hydrocodone that is also utilized clinically as an analgesic. Carisoprodol is used primarily as a centrally acting muscle relaxant with meprobamate being its primary metabolite. Meprobamate is also utilized clinically as a sedative, anxiolytic, and muscle relaxant. Cyclobenzaprine is a tricyclic compound that is structurally similar to amitriptyline but is utilized as a centrally acting muscle relaxant. Some of the more obvious effects of these CNS depressant drugs such as slurred speech, drowsiness, dazed appearance, diminished ability to concentrate or multi-task, and confusion are demonstrated on the video. Additionally, cyclobenzaprine is known to have some anti-histaminic activity which can result in dry mouth, which is also demonstrated on the video.

Drug Impaired Driving, CNS Depressants, Urine

K28 Driving Under the Influence (DUI) in Southern Ohio — Drug Demographics for the Drugs Encountered in DUI Case Work

Laureen J. Marinetti, PhD, Montgomery County Coroner's Office, Miami Valley Regional Crime Lab, 361 West Third Street, Dayton, OH 45402*

After attending this presentation, attendees will become aware of the drug demographics seen in DUI cases analyzed in a regional crime laboratory in Ohio. The region covers a radius of approximately 75 miles around the city of Dayton. This presentation will be an overview of the most commonly encountered drugs in DUI cases analyzed at the MVRCL. Drugs that were encountered in 817 DUI cases during 2005 will be reviewed. Case examples will be used and quantitative values in blood will be listed when available.

This presentation will impact the forensic community and/or humanity by making data available as to commonly encountered drugs in a specific region of Ohio to be used by other labs analyzing DUI cases in making changes to screening protocol or changes to the testing approach used in detecting these drugs. Finally, the top thirteen drug classes will be reviewed in detail as well as mention made of Ohio's new per se law outlining per se levels for marijuana, marijuana metabolite, cocaine, cocaine metabolite, heroin, 6-monoacetylmorphine, amphetamine, methamphetamine, lysergic acid diethyl amide, and phencyclidine in blood, serum plasma and urine Mention will also be made as to how the drugs chosen in this law may bias some laboratories' DUI protocol.

Methods: DUI cases are first subject to quantitative ethanol analysis by headspace gas chromatography. Depending upon the ethanol result and the case history, analysis may stop or continue for drug analysis. Analysis proceeds with enzyme linked immunosorbent assays (ELISA) for the following drugs or drug classes with cut-offs in blood and urine listed (ng/mL): amphetamine (50), barbiturates (500), benzodiazepines (10), cannabinoids (20), carisoprodol (1000), cocaine metabolite (100), methamphetamine (50), and opiates (25). Any positive ELISA results are subject to confirmation by gas chromatography with mass spectral, flame ionization, nitrogen phosphorus, or electron capture detection. If there are no positive ELISA screens, the case may be subject to a variety of analyses depending upon the amount of specimen submitted and the case history. These analyses can include, but are not limited to: benzodiazepines by gas chromatography with electron capture detection, basic, acidic and neutral drug screens by gas chromatography mass spectrometry (GC/MS), GHB and 4-methyl GHB by GC/MS, sympathomimetics by GC/MS, gabapentin and baclofen by high performance liquid chromatography with diode array detection, and additional ELISA screens for fentanyl (1), phencyclidine (5), and oxycodone (25).

Results: The most commonly encountered drugs (occurrence of 10 or greater) are listed by class in the table below. The drugs are listed as the number of occurrences because many cases involved multiple drug/ethanol findings. The results are further broken down by occurrences of each drug individually. Opiate occurrences were hydrocodone 67, oxycodone 59, morphine 49, codeine 28, and hydromorphone 4. Benzodiazepine occurrences were alprazolam 110, clonazepam/7-aminoclonazepam 33, diazepam/nordiazepam 29, temazepam 9, oxazepam 5, lorazepam 4, midazolam 2, and triazolam 1. Antihistamine occurrences were promethazine 7, dextromethorphan 7, chlorpheniramine 4, diphenhydramine 4, orphenadrine 3 and, doxylamine 3. Analgesic occurrences were: propoxyphene/norpropoxyphene 7, gabapentin 6, fentanyl 4, tramadol 4, trazodone 2, and meperidine 1. Antidepressant occurrences were amitriptyline/nortriptyline 4, citalopram 4, fluoxetine 4, sertraline 3, bupropion 3, and venlafaxine 3. Barbiturate occurrences were butalbital 11. Others drug classes that were confirmed included: hypnotics - zolpidem 8; sympathomimetics – methylenedioxymethamphetamine/ methylenedioxyamphetamine 2, and phentermine 1; muscle relaxants – cyclobenzaprine 3, metaxalone 1, and methocarbamol 1; antipsychotics – mirtazapine 1; anticonvulsants – phenytoin 2, and topiramate 1.

	Drug Class	Number of Occurrences
1	Ethanol	446
2	Cannabinoids	230
3	Opiates	201
4	Benzodiazepines	158
5	Cocaine	111
6	Pseudoephedrine/Ephedrine	53
7	Carisoprodol/Meprobamate	29
8	Antihistamines	28
9	Methadone	24
10	Analgesics other than opiates	24
11	Antidepressants	21
12	Amphetamine/Methamphetamine	21
13	Barbiturates	11

Summary: The data show that a great majority of the drugs responsible for DUI cases in Ohio are not necessarily illicit drugs. With the exception of ethanol, law enforcement and legislators continue to focus their efforts on illicit drugs as far as improved legislation and control. The new Ohio per se law is a perfect example of this mindset. This law defines per se levels for amphetamine, cocaine, cocaine metabolite, heroin, 6-monoacetylmorphine, lysergic acid diethylamide (LSD), marijuana, marijuana metabolite, methamphetamine, and phencyclidine (PCP) in blood, serum, plasma, and urine. As reflected in the data above, only the cocaine and metabolite and the marijuana and metabolite play a significant role in DUI. For 2005, MVRCL had no positive PCP cases, no driving histories consistent with LSD use, and few amphetamine/methamphetamine positives compared to the benzodiazepine and opiate classes. Intact heroin is never detected in an ante-mortem biological specimen. A per se level for any drug in urine is not meaningful as far as supporting a direct relationship between the drug and the impaired driving at the time of the offense. Ohio does not utilize the drug recognition expert program. Therefore, based on the law, urine per se levels can legally stand on their own, independent of field sobriety tests or any other measurement of impairment. Although the committee that drafted this legislation had ample consultation with toxicologists from all over the state, the toxicologists' recommendations were largely ignored. Because of the new Ohio law and others like it, some laboratories may be tempted to concentrate on those drugs with per se levels and ignore the rest. As demonstrated by the data, adopting this practice would potentially miss a majority of the drugs responsible for altered driving in DUI cases.

DUI, Drug Per Se Level, Demographics

K29 DUID Case Studies — DRE Evaluations With Blood / Oral Fluid Drug Quarts

Colleen E. Scarneo, BS, and Michael A. Wagner, PhD, Department of Safety - State Police Forensic Laboratory, 33 Hazen Drive, Concord, NH 03305*

After attending this presentation, attendees will: 1) appreciate the important factors used to evaluate a potential drug impaired driving case, including the role of a DRE officer, 2) learn about the pharmacology of specific benzodiazepines and opiates and their effects on driving, 3) consider oral fluid as a possible alternative matrix to blood or urine in DUI investigations based upon the strengths and weaknesses discussed.

This presentation will impact the forensic community and/or humanity by building a database of drug quantitations in blood to relate to driving impairment.

Drug impaired driving continues to be a societal problem and growing concern as the number of physician written prescriptions and

illicit drug use increase. Polydrug use further complicates the issue by making interpretation more difficult, frequently as the result of insufficient information to support an opinion. Unlike alcohol, drug concentrations are not correlative to behavioral effects, particularly in DUI cases. Furthermore, limited literature information is available correlating drug concentrations with standardized field sobriety tests and poor driving performance. The following cases are presented to support the need for more case specific data correlating drug concentrations to driving performance.

The first case involves a 43-year-old male charged with DWI (2nd offense) after a witness reported observing the suspect driving erratically on a major highway. The suspect failed the standardized field sobriety tests (SFSTs) administered by the arresting officer. The suspect claimed to suffer from chronic back pain, and several medications were seized from him including Oxycontin® (40 mg), diazepam (10 mg), Skelaxin® (metaxalone 800 mg), Lyrica® (pregabalin 75 mg), as well as other drugs that were not readily identified. The breath alcohol test was negative, so a DRE was summoned. Upon completion of the DRE evaluation, the officer opineed that the suspect was under the influence of a CNS depressant and narcotic analgesic. Blood and urine samples were tested. The laboratory quantitatively determined diazepam (410 ng/mL), nordiazepam (481 ng/mL), oxazepam (48 ng/mL), and trace amounts of temazepam, as well as oxycodone (114 ng/mL). The laboratory did not test for metaxalone or pregabalin. The urine sample was presumptively positive for benzodiazepines, opiates, cannabinoids, and cocaine.

The second case involves a 38-year-old male nurse charged with DUI after a witness complained of his erratic driving to state police. The arresting trooper was also able to observe the driver's dangerous behavior while driving and pulled him over soon after. Upon initial contact, the driver was observed to be wearing his coat inside out and upside down. A recently filled prescription for lorazepam (0.5 mg) fell out of the suspect's pocket. The suspect failed initial SFSTs and the breath alcohol was negative, so a DRE was called. The DRE opined that the suspect was under the influence of a CNS depressant and cannabis. The suspect admitted to taking lorazepam on an "as needed" basis, but did not take it regularly. Physician affidavits were obtained verifying that the suspect was under their medical care and prescribed Ativan® and Seroquel®. The laboratory's findings reflected a blood quantitation of lorazepam (79 ng/mL). The laboratory did not test for the Seroquel®. The presumptive positive cannabinoids drug screen was subsequently confirmed negative for both THC and THC-COOH.

The last case involves a subject, who voluntarily participated in an ongoing study evaluating the applicability of oral fluids to DRE certifications/DUI investigations. Oral fluid testing is not new to forensic toxicology; however, the use of oral fluids in DUI cases is being developed. Oral fluids offer many potential advantages over conventional blood and urine matrix testing, particularly the ease of sample collection. Blood, urine, and oral fluid samples are collected from the volunteer who is under the influence of CNS depressants and narcotic analgesics. The results of each matrix are compared to one another and against the DRE's opinion.

All three cases reflect the laboratories' limitations in terms of the types of drugs tested and matrices used. Forensic Toxicology is an ever-expanding field that must consider ways to optimize and standardize testing through collaborative research and sharing of data.

DRE, Impaired Driving, Forensic Toxicology

K30 Dissociative Driving: Ketamine DUID Fatality Case Study

Kevin M. Lougee, BS, Amy L. Lais, BS, Kim G. McCall-Tackett, BS, Diane J. Mertens-Maxham, BS, R.E. Kohlmeier, MD, and Norman A. Wade, MS, Maricopa Country Medical Examiner, 701 West Jefferson Street, Phoenix, AZ 85007*

After attending this presentation, attendees will understand the pharmacological effects of ketamine and how it may adversely affect driving tasks.

This presentation will impact the forensic community and/or humanity by demonstrating the adverse effects of ketamine on driving skills, motor performance, and behavior in an otherwise healthy individual.

Ketamine came into existence as a safer alternative to PCP. Even before PCP was withdrawn from the market due to its problematic adverse reactions in patients, pharmaceutical houses were looking for a safer alternative that would have less toxic behavioral effects. It was first synthesized in 1962 and patented in 1966 under the trade name Ketalar® and received FDA approval in 1970 as a general anesthetic. It is used as a short-acting induction anesthetic that provides a profound, rapid, dissociative anesthesia and a short recovery time. Low doses produce effects similar to PCP but doses in the anesthetic range (1mg/kg) produce experiences where the individual feels separated from his body, floating above his body and a near-death experience. This state, which users call the "K-hole," can either be spiritually uplifting or terrifying (heaven or hell). Ketamine is a synthetic, sedative, non-barbiturate that acts as a central nervous system depressant and produces a rapid-acting dissociative effect. It is used in the recreational drug market by illegally diverting from legitimate suppliers, allowing the liquid carrier to evaporate. The crystals are scraped into a fine powder and packaged. The first reports of ketamine abuse occurred in the early 1970s in the San Francisco and Los Angeles areas.

In this case report, a 27-year-old Caucasian male was the driver and sole occupant of a luxury sedan driving on a dry and clear Arizona freeway at two thirty AM on an early February morning. He attempted to exit this freeway for an unknown reason; however, in doing so, he hit a traffic sign and then continued about 1100 feet until colliding head-on with a large steel sign post. The force of the collision caused the vehicle to rotate 180 degrees and caused very extensive front end damage with major intrusion into the cab area of the automobile. It was reported that the decedent was not wearing a seat belt and was not exceeding the posted 65 miles per hour speed limit.

A full autopsy was performed approximately 48 hours after death was pronounced and cause of death was determined to be massive blunt force trauma due to head and neck (fractured and dislocated) injuries. These included transection of the cervical spine, laceration of the pericardial sac, transection of the thoracic aorta, bilateral rib fractures, and contusions of all lobes of the lungs and multiple splenic lacerations. The manner of death was accident. During autopsy the assistant medical examiner collected pleural blood, bile, vitreous and gastric contents for complete toxicological testing. Vitreous and blood were analyzed for volatiles by GC-FID while the pleural blood was assayed by ELISA for benzodiazepines, barbiturates, benzoylecgonine, opiates, and methamphetamine with negative results. The blood and bile specimens were subjected to a qualitative analysis for basic drugs, and ketamine and its metabolites were confirmed by GC/MS using electron impact ionization. Quantitation of the ketamine was performed on the pleural blood with the result being 1.5 mg/L of parent compound. Further quantitative testing of all tissues submitted will also be presented.

A presentation of this case study will contribute to establishing guidelines on potential impairment concentrations of ketamine as it relates to DUID cases. Although this case demonstrates only anecdotal evidence for DUI impairment, it clearly demonstrates the adverse effects of ketamine on driving skills.

Ketamine, DUID, Fatality

K31 Methadone and Impaired Driving

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After attending this presentation, attendees will learn about the growth in the incidence of methadone in death investigation and impaired driving casework and some of the considerations in presenting methadone evidence in court.

This presentation will impact the forensic community and/or humanity by demonstrating how methadone is increasingly encountered in the forensic toxicology community and in the population of drivers arrested for DUI drugs. The relevant literature has few references to methadone-impaired driving and this data will be valuable to practitioners encountering the methadone-impaired driver.

The Washington State Toxicology Laboratory performs analysis in death investigation and criminal cases. It has previously reported an increased incidence of methadone[1] in casework, and the rise in both the number of methadone positive cases and the overall percentage of cases received has continued. In 1993, the laboratory reported 20 positive methadone cases which represented less than 0.5% of the casework. In 2005, this had risen to 640 methadone positive cases representing 6.7% of all cases received. Of these, 237 were drivers arrested for investigation of Driving Under the Influence (DUI). These increases are due in part to an increased use of methadone prescriptions for chronic pain management. Laboratory staff are often asked to evaluate the role of methadone in impaired drivers, which is often complicated by polypharmacy. This report will review the demographics, performance and behavior, and toxicology findings in a series of drivers arrested under investigation of DUI.

There are reports in the literature suggesting that methadone does not cause impairment among patients on a stabilized methadone dose[2]. Baselt states, "narcotic–tolerant subjects can be stabilized on methadone replacement therapy with few subjective or objective effects on performance"[3]. The current study was undertaken to evaluate the role of methadone in impaired drivers. Toxicology reports of methadone positive drivers arrested for DUI from 2000 through 2005 were reviewed. There were 629 subjects, 62% of whom were males, mean and median age of 41 and 42, respectively with a mean and median methadone concentration of 0.21 and 0.15 mg/L. Methadone was rarely the only significant finding in these cases; approximately 98% of drivers are positive for at least one other psychoactive substance besides methadone, which is comparable to a rate of 92% in post-mortem cases. This group included 32 cases where methadone was the only psychoactive drug present in the suspect and where the subject was evaluated by a Drug Recognition Expert (DRE). Of this group, 75% were males, mean and median age was 40 and 42 respectively, and the mean and median methadone concentrations were 0.26 and 0.27 mg/L. Forty-two per cent were involved in a collision and 45% were stopped for erratic lane travel. A third of the erratic drivers were weaving so severely, that cell phone callers notified police of the potential DUI. The collisions most often involved striking parked vehicles, in one case the driver, while attempting to park drove up onto a curb, "deep-trunked" a parked vehicle, backed up and drove over the curb again.

The observations made on these subjects were compared with the signs and symptoms associated with narcotic analgesics from the DRE examination. Pupil sizes were consistently constricted in room light, near darkness, and in direct lighting conditions; there was little to no reaction to light and muscle tone was described as flaccid. These observations were consistent with the DRE matrix. Blood pressure and pulse rate varied widely, while the DRE matrix predicts that they would both be below the normal range. On the psychophysical tests, the subjects averaged 5/8 on the walk and turn, 3/4 on the one leg stand and 2" of sway on the Romberg balance. Time estimate was not a consistent marker for methadone impairment but they generally performed poorly on the modified finger to nose test. Approximately half of the subjects had slurred speech and 75% were described as having droopy eyelids.

In one case, a 35-year-old male collided with another vehicle in the same lane of travel. When officers arrived at the scene, the subject was seated in his vehicle and appeared to be "nodding off." The subject voluntarily performed the standard field sobriety tests for the responding officer after which a DRE officer was called to the scene. The DRE officer noted that the subject had slurred speech, watery eyes, and droopy eyelids. He continued to "nod off" during his transport to the local precinct. He also repeatedly asked the same questions of the officer throughout the evaluation, without recognizing that he asked the same questions previously. On the Romberg balance he exhibited 2 to 3 inches of sway and he asked to repeat the test 3 times with his 30 second time estimates being 36, 45 10 and 76 seconds, respectively. He exhibited 6/8 clues on the Walk and Turn with his legs shaking throughout the test. His legs also shook on the One Leg Stand and he exhibited 3/4 clues. He only had one correct touch (of six attempts) on the Modified Finger to Nose. He did not exhibit Horizontal Gaze Nystagmus (HGN), Vertical Gaze Nystagmus (VGN), or a lack of convergence. His pupil sizes were 2.5 mm in room light (within normal range); 3.0 mm in darkness (below normal range) and 2 mm in direct light (within normal range) and he showed very little reaction to light. His pulse rate was elevated (100, 108 and 106) and his blood pressure was elevated 172/90. His muscle tone was described as rigid. He admitted to 30 mg of Methadone for chronic pain approximately 3.5 hours before the collision. The subject was arrested for DUI-drugs and his blood toxicology report was positive for methadone, at 0.27 mg/L, EDDP - methadone metabolite, nicotine, and caffeine.

Seventy-eight per cent of these subjects admitted to methadone use, with 31% indicating they were participants in an Opiate Treatment Program (OTP) and 34% indicating they were taking methadone for chronic pain treatment.

The data support the position that methadone can impair driving both for subjects in opiate treatment programs and for patients receiving it for treatment of chronic pain, and provide some parameters for comparison without complication of co-ingestion from other impairing drugs.

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Impaired Driving, Methadone, Drug Recognition Expert (DRE)

K32 Altered Pharmacokinetics of Delta 9-tetrahydrocannabinol

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The goal of this presentation is to provide forensic toxicologists and pathologists additional factors to consider in interpreting drug levels following traumatic injuries.

This presentation will impact the forensic community and/or humanity by helping in the understanding of the relationship between delta-9-tetrahydrocannabinol (THC) and driving impairment;

understand pharmacokinetics of THC; and understand some factors that could affect THC metabolism.

Marijuana is the common name for a crude drug made from the plant Cannabis sativa. It is commonly used for its euphoric effects. The main mind-altering (psychoactive) ingredient in marijuana is delta-9-tetrahydrocannabinol (THC). Some immediate physical effects of marijuana include a faster heartbeat and pulse rate, bloodshot eyes, and a dry mouth and throat. Smoking marijuana results in rapid absorption and is measurable in plasma within seconds after inhalation. Peak THC plasma concentrations occur prior to the end of smoking, and vary depending on the potency of marijuana and the manner in which the drug is smoked. In general, peak THC serum levels typically exceed 100 ng/mL and then fall rapidly: within 2 hours plasma THC concentrations are at or below 5 ng/mL. As a result, detection times in the blood are typically only a few hours after past use. THC has a large volume of distributions and is highly protein bound. THC is eliminated from plasma in a multiphasic manner. Metabolism of THC occurs via the hepatic Cytochrome P450 enzyme system. The major metabolite is 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH).

Marijuana has been shown to impair performance on driving simulator tasks and on open and closed driving courses for up to approximately four hours. Decreased car handling performance, increased reaction times, impaired time, and distance estimation, inability to maintain headway, lateral travel, subjective sleepiness, motor incoordination, and impaired sustained vigilance have all been reported. In 2004 in British Columbia, Canada approximately 25% of drivers involved in fatal accidents had evidence of marijuana use as determined by blood and urine detection of THC or THC-COOH.

A 16y old male driver presented in the emergency department with multiple blunt force injuries sustained during a single vehicle crash. Plasma samples that were collected in the ED for analysis had been determined to have concentrations of THC of 460 nmol/L (0.14mg/L), and a THC-COOH of 110 nmol/L (0.038 mg/L). The individual stated that he smoked marijuana prior to the incident, but that it was a couple hours prior to the sample collection. Based on the THC and THC-COOH concentrations using previously published algorithms, it is predicted that marijuana was smoked less that one hour prior to the sample collection. This contradicts the individual's claims.

Many physiological factors lead to altered pharmacokinetics parameters of commonly prescribed medications, including shock, altered blood flow, and altered perfusion of vital organs. The case demonstrated altered THC pharmacokinetics potentially due to traumatic injuries sustained by the individual.

Driving Impairment, Delta-9-tetrahydrocannabinol (THC), Pharmacokinetics

K33 Methamphetamine Impaired Driving in Arizona

John J. Musselman, BS, and Anil Solanky, MS, Phoenix Police Department Crime Laboratory, 620 West Washington, Phoenix, AZ 85003*

After attending this presentation, attendees will learn of the harmful combination of the methamphetamine lifestyle and driving.

This presentation will impact the forensic community and/or humanity by exposing common driving errors, individual behavior and observations, drug recognition expert (DRE) evaluations, and quantitation of amphetamine and methamphetamine in DUID cases. Specific case studies will be presented.

Although ethanol impairment continues to dominate in the workload, CNS stimulants, primarily methamphetamine has become the second most common drug(s) found in both blood and urine of drivers cited in driving under the influence of drugs (DUID). Urine is collected in misdemeanor DUID cases and blood is collected in more serious felonies and fatalities when impairment is suspected. Vehicular behavior and/or the reason for stop prior to evaluation can be more difficult to obtain. Furthermore, evaluation of an individual by a DRE can be limited due to refusal, uncooperative, combative, or injured person. Sometimes vehicular behavior is the best indicator of impairment; sometimes it is the only indicator. Obtaining accident reports, field officer's notes and DRE evaluations and collation of the information can be a very useful tool when an opinion on impairment is asked of the toxicologist.

Amphetamine, methamphetamine were analyzed quantitatively as TFA derivatives with d5 amphetamine/d9 methamphetamine as internal standards. A review of 31 cases in 2004 where methamphetamine was the only significant finding, the average amphetamine concentration was 49ng/mL (median 33, range 10-160) and methamphetamine was 470ng/mL (median 330, range 63-1700). A breakdown of the blood methamphetamine levels; twelve (38%) were<200 ng/mL (considered "therapeutic"), and four (13%) were>1000 ng/mL. Acetone was qualitatively identified in the blood in three (10%) of the samples. When analyzing samples for blood ETOH, acetone and the absence of ethanol in DUI cases was a good indicator of CNS stimulant use.

Amphetamine/Methamphetamine (A/M) ratios ranged from 2-38%, with only four cases (13%) having (A/M) ratio <5%, indicative of an acute dose. Laboratory data suggests that the majority of these individuals are likely impaired due to the "down-side" or withdrawal effects of methamphetamine.

Methamphetamine users are mostly white males. A high percentage (approx 50%) of users are unemployed, have a suspended or no driver's license, no insurance, etc. The most common drugs found with samples containing methamphetamine are in decreasing order THC, benzodiazepines, and cocaine/BE. While DUI Ethanol arrests occur 80% of the time between the hours of 8:00 pm to 4:00 am, methamphetamine users are apprehended around the clock. Driving infractions included speeding, erratic driving, cross-center line collisions, red light, rear-end collisions, asleep at the wheel, and stolen vehicle and/or plates. Observations by officers (not necessarily DREs) include blood-shot watery eyes; slurred, mumbled, or incoherent speech; restlessness and body tremors. Evaluations performed by DREs demonstrated the best indicators of stimulant use were; the lack of horizontal gaze nystagmus (HGN), body tremors, pulse (n=14) mean 99 (60-136), and pupil measurement in dark room (n=5) mean 7.6 mm. Data from 2005 is currently being collected and will be presented, along with case studies.

Methamphetamine, Driving, Impairment

K34 Discovery and the Forensic Toxicology Laboratory

E. Warren Street, JD, Laboratory Corporation of America, 7777 Forest Lane, Suite C350, Dallas, TX 75230*

After attending this presentation, attendees will understand which provisions of the voluminous Federal Rules of Civil and Criminal Procedure directly apply to them in their routine production and retention of data, records, and documentation.

This presentation will impact the forensic community and/or humanity by identifying the specific rules of discovery that obligate the forensic scientist to maintain forensically defensible records of their work. The focus will be on those rules that may conceal inherent legal pitfalls for the prosecutor, plaintiff, or defendant who is not diligent in their request for the production of documents and witnesses.

The Federal Rules of Civil Procedure (FRCP) were promulgated by The United States Supreme Court. They were subsequently modified, ratified, and adopted by the Congress in 1938. Rule 16 of the Federal Rules

of Criminal Procedure was likewise adopted in 1946. During the intervening years the Rules have been further modified, amended, and expanded.

The term "discovery" encompasses the methods by which a party or a potential party to a lawsuit or prosecution obtains and preserves information regarding the action.

The Federal Rules of Civil and Criminal Procedure specify in considerable detail the means by which discovery may be used by plaintiffs, prosecutors, and defendants to compel the production of such data, witnesses, and documents as are needed at trial. It will also be emphasized that, while most states have adopted part or most of the Federal Rules, due diligence is essential in determining what deviation from the Federal Rules exists in a given jurisdiction, if the litigation is going to be heard in a state court.

Discovery in Civil matters is controlled by the Federal Rules of Civil Procedure, Rules 26 through 32. Discovery in criminal matters, greatly restricted by the constitutional protection against self-incrimination on the part of the defendant, is controlled by the Federal Rules of Criminal Procedure Rules 15 through 17 and Rule 26.2. Those instances where certain Federal Rules of Evidence are closely linked to the procedural rules will also be covered.

In civil matters, the duty to disclosure is essentially equal between the parties. This presentation will closely examine FRCP Rule 26 in particular and how it relates to the retention of experts. It will provide clear distinction between the consulting expert and the testifying expert in their obligation to disclose information.

Federal Rules, Discovery, Scientific Documents

K35 Discovery Issues: Deposing the Forensic Toxicologist

Harry L. Miles, JD, Green, Miles, Lipton, & Fitz-Gibbon, LLP, 77 Pleasant Street, PO Box 210, Northampton, MA 01096*

After attending this presentation, attendees will learn how to help the attorneys who engage them prepare them for written discovery requests, depositions and how to respond to discovery requests and questions so that the truth prevails. The presentation will aid the forensic community by helping the American justice system find the truth in contested criminal and civil matters. The presentation will focus upon the obligations of the experts and the attorneys to zealously represent their clients' interests without violating their ethical obligations.

This presentation will impact the forensic community and/or humanity by demonstrating the practical preparation needs and potential pitfalls discovery may impose upon a toxicologist.

State and Federal rules of civil and criminal procedure impose discovery obligations on each party. Experts may have trouble reconciling their professional ethics with discovery practices under the rules of procedure. The presentation will discuss the practical needs of attorneys for expert opinions and the ways in which experts can provide those opinions in good conscience. In addition, the presentation will inform the attendees of the tricks, traps and pitfalls they may encounter when they respond to written discovery requests or testify at depositions. The manner in which deposition testimony may be used against an expert who testifies at trial will be demonstrated.

Discovery, Experts, Ethics

K36 The Role of the Forensic Expert in the Discovery Deposition: Communication is Key

David M. Benjamin, PhD, 77 Florence Street, Suite 107, Chestnut Hill, MA 02467*

The goals of this presentation are to review rules of evidence and procedure essential to quashing abusive discovery requests, develop the ability to actively listen to questions, recognize unclear questions, and practice asking for clarification.

This presentation will impact the forensic community and/or humanity by empowering forensic experts with information about how to prepare for a deposition, and providing instruction on the importance of listening to every word in a question before answering.

The "Discovery" phase of litigation is designed for each party to inquire about various positions of the other side and request production of important documents. After documents have been exchanged, experts may be asked to prepare a report memorializing their findings and opinions. The parties exchange expert reports and in jurisdictions where expert depositions are allowed, each party may elect to take the deposition of one or more of the opposing experts. A deposition is sworn testimony outside the presence of a judge and jury. Any fact witness or expert who has been disclosed (designated to be called as a witness at trial) may be asked to submit to a deposition. If you are to be deposed, you will probably receive a subpoena *duces tecum* asking you to bring certain documents to your deposition. Responding to the subpoena requires collaboration between the deponent and the attorney who retained him/her in order to determine the propriety of the requested documents.

Testifying as an expert in toxicology, or preparing your client for his/her deposition involves not only a thorough review of the facts of the case, but the ability to report one's findings in an oral question and answer format. To be an effective witness, the deponent (individual being deposed) must be able to wade his/her way through the confrontational mine field of rhetoric and subterfuge that pervades much "lawyer-talk," learn to recognize, and respond to trick questions. While experts have spent decades becoming proficient in their areas of specialty, attorneys have spent years learning how to phrase questions designed to elicit admissions and concessions, or to make you look foolish or unqualified.

This presentation is designed to teach you to develop "Active Listening" skills you can use to recognize improper or poorly framed questions, and to empower you with insights into the deposition process that will permit you to avoid providing an even worse response to a poorly phrased question. The seminar will begin with a review of the roles an expert can play in the legal system and continue with a brief review of the rules of evidence and civil procedure which control discovery requests, subpoenas, and provide for limiting and quashing abusive discovery requests.

The workshop will proceed to examine the objectives of a deposition and what types of questions to anticipate. Examples of good and bad deposition testimony and a review of an excellent deposition instruction provided by one attorney to a deponent will be presented.

Responding to Subpoenas, Preparing for Your Deposition, Developing Active Listening Skills

K37 A Rapid Increase in Fentanyl-Related Deaths in Detroit — A Twelve Month Review

Daniel S. Isenschmid, PhD, Bradford R. Hepler, PhD, Denice M. Teem, BS, and Carl J. Schmidt, MD, Wayne County Medical Examiner's Office, 1300 East Warren Avenue, Detroit, MI 48207*

After attending this presentation, attendees will be aware of the statistics associated with a large increase in fentanyl-related deaths in the Detroit area in the past year.

This presentation will impact the forensic community and/or humanity by raising awareness of a public health issue related to the dangers in the use of street drugs and by making the toxicologist aware of the need to include fentanyl in their drug abuse screens.

Beginning in late August 2005 there was a rapid increase in fentanyl-related deaths observed by the Wayne County Medical Examiner's Office (WCMEO). Most of these deaths were also associated with cocaine and/or heroin use. A year later there have been a total of 132 deaths associated with fentanyl in combination with these drugs.

Reports from crime laboratories from the analysis of exhibits and seizures from clandestine laboratories suggested that illicit fentanyl was being manufactured and sold by itself or mixed with heroin or cocaine. Street names for the drug combinations have included "suicide," "drop dead", "reaper", "penicillin", "lethal injection" or "crazy" stamped on the packets.

The WCMEO has routinely looked for fentanyl in its GC/MS screen (LOD 5 ng/mL) since 1999 in cases pending for toxicology and deaths due to accident or suicide. Prior to 2002, the incidence of fentanyl never exceeded 10 per year. Due largely to increased use and abuse of fentanyl patches and lollipops the incidence of fentanyl (defined as confirmed in any specimen) in postmortem examinations has gradually increased to 12 (2002), 20 (2003), and 29 (2004). For the first 8 months of 2005 there was an incidence of 15 cases in which fentanyl was detected, but by the end of the year that number rose to 63. For the first eight months of 2006 the incidence surged to 159. Due to the large increase in fentanyl associated cases, the WCMEO has since instituted a blood fentanyl screen by ELISA (Immunalysis®, Pomona, CA). This allows the laboratory to perform a rapid screen for the drug in more cases than before using a cutoff of 2 ng/mL. The assay shows good separation around the cutoff when using controls at 1 and 4 ng/mL.

Since May 2005, confirmation and quantitation of fentanyl has been performed at the WCMEO using GC/MS SIM (LOD = 1 ng/mL). Prior to that date, fentanyl was quantitated at a referral laboratory. Table 1 shows a statistical break down of the fentanyl concentrations determined in cases attributed to the fentanyl-laced deaths. In most cases heart blood was available. When iliac blood was available it was analyzed and the heart blood to peripheral blood ratio was calculated. Some of these ratios were quite high. Although post-mortem redistribution of fentanyl is possible, some of these deaths involved finding the decedent with a syringe still in the arm or groin, suggesting that early, partial drug distribution may also play a role in some high blood to peripheral blood ratios.

Table 1: Fentanyl concentrations (ng/mL) and Heart / Peripheral concentration ratios

	Heart Blood	Iliac Blood	Ratio Heart/ Iliac
Mean	33	17	2.7
Median	23	14	2.0
Range	3 – 190	3 – 69	0.47 – 10.7
N	155	85	80

From the first combined drug death on August 28, 2005 – August 31, 2006 the following number of cases were reported: fentanyl and heroin (as 6-acetylmorphine in blood or vitreous humor) (50), fentanyl and morphine (suspected heroin) (5), fentanyl and cocaine (includes benzoylecgonine) (36), fentanyl, heroin, and cocaine (41). Excluding hospital-administered fentanyl, another 41 deaths were due to fentanyl intoxication by itself or in combination with other prescription drugs. The fentanyl in most of these cases was suspected to be from illicit sources although a few of these cases included patch abuse and lollipop abuse. The overall incidence of ethyl alcohol in these cases is quite low (21%).

The demographics of the population were interesting and suggested widespread use of the combination of drugs throughout the metropolitan area. The WCEMO serves all of Wayne County (population 2 million) which includes Detroit (population 885,000). In the 2005 census, the population of Wayne County as a whole was 54% white and 42% black with City of Detroit population being 12% white and 82% black. For the fentanyl-related deaths the majority of decedents were male (67%), white (62%), were non-Detroit residents (53%), and between 40-59 years of age (64%). Only five cases were under the age of 20 although 17% of cases were between 20 and 29 years of age. Most were found dead at the scene (35%) or in their home (41%). Relatively few made it to hospital and many were dead on arrival.

In most cases, the medical examiners have reported the cause of death by listing the drugs present (e.g. cocaine, heroin and fentanyl intoxication) although if additional prescription drugs were present they may report the cases as multiple drug intoxication. The manner of death was accident in all of the cases.

After excluding hospital administered fentanyl, the relative lethality of fentanyl (defined as the percent of cases signed out as a drug related death when the drug is present) is exceeded only by carbon monoxide when present in blood. For all cases in which fentanyl was present acutely in addition to cocaine and / or heroin during this 12-month study period, only one case, a pedestrian, did not die directly from the drug combination.

Fentanyl, Heroin and Cocaine, Deaths

K38 Two Pediatric Methadone Fatalities: Case Reports From the Office of the Medical Examiner, Phoenix, Arizona

Kim G. McCall-Tackett, BS, Amy L. Lais, BS, Diane J. Mertens-Maxham, BS, Vladimir Shvarts, MD, and Norman A. Wade, MS, Maricopa County Medical Examiner, 701 West Jefferson Street, Phoenix, AZ 85007*

After attending this presentation, attendees will be afforded a review of two recent pediatric methadone related deaths.

This presentation will impact the forensic community and/or humanity by demonstrating that these analytical results will contribute to the limited data available on pediatric methadone cases.

The authors will present information about two recent pediatric deaths attributed to methadone toxicity. The analytical results presented are significant for their contribution to the limited data on methadone in a pediatric population.

Methadone, a synthetic opioid, was first synthesized by German scientists during World War II. It became clinically available in the United States in 1947 for treatment of narcotic addictions and later used for the treatment of chronic pain. Despite concern about the increased prescription of methadone and methadone related deaths, there is little information about incidence and associated toxicity in children. Prior use and cellular tolerance is of particular significance in evaluating upload blood concentrations. As documented in the literature, blood concentrations in a fatal overdose can vary greatly, with overlapping therapeutic and lethal ranges.

Case #1: A 12-year-old female was found unresponsive in her bedroom by family members. Emergency medical personnel were summoned, but resuscitation efforts were unsuccessful and death was pronounced at the scene. A full autopsy was performed approximately 55 hours after death was pronounced. Significant findings were elevated lung and brain weights consistent with edema. Pleural fluid, bile, vitreous fluid, gastric contents, liver, spleen, and brain were collected and submitted for toxicological analysis. Volatiles were assayed by GC-FID. Ethanol was present in the vitreous fluid at 0.01 g% and 0.07 g% in the pleural fluid. The blood was screened by ELISA for benzodiazepines, barbiturates, benzoylecgonine, opiates, and methamphetamine with negative results. The blood and bile specimens were subjected to a qualitative analysis for basic drugs. Methadone and metabolites were confirmed by GC/MS. The methadone was quantitated by GC-NPD in all specimens with results as follows: Pleural fluid 0.70 mg/L, liver 5.98 mg/kg, spleen 3.43 mg/kg, brain 1.15 mg/kg, and gastric contents 111.27 mg/L. The cause of death was determined to be methadone toxicity, and the manner of death was ruled a suicide.

Case#2: A 5-year-old male was found unresponsive in bed by his father. Emergency medical personnel were summoned, but resuscitation efforts were unsuccessful, and death was pronounced at the scene. A white foam cone from the nose and a reddish purge from the mouth were noted by medical examiner personnel. The decedent's prior medical history includes recent fever and congestion, autism, and asthma. A full autopsy was performed approximately 24 hours after death was pronounced. Significant findings include marked cerebral edema, heavy lungs, and an enlarged heart. At autopsy, cardiac blood, urine, bile, vitreous fluid, and gastric contents were collected and submitted for toxicological analysis. Volatiles were assayed by GC-FID with negative results. The blood was screened by ELISA for benzodiazepines, barbiturates, benzoylecgonine, opiates, and methamphetamine with negative results. The blood and urine specimens were then subjected to a qualitative analysis for basic drugs. The analytical findings are as follows: Methadone and metabolites were confirmed by GC/MS. The methadone was quantitated by GC-NPD and the concentration was found to be: Cardiac blood 0.34 mg/L, urine 8.36 mg/L, bile 0.63 mg/L, and gastric contents 23.8 mg/L. Also present in the cardiac blood were: Doxylamine 0.36 mg/L, Dextromethorphan 0.24 mg/L, and Acetaminophen 69.0 mg/L. The cause and manner of death are pending in this case, as it is still under investigation.

In both cases, a quantitative analysis of methadone consisted of a basic pH butyl chloride extract of sample and mepivacaine internal standard analyzed on an Aglient 6890 GC equipped with a nitrogen phosphorous detector (NPD). Analytical conditions consisted of a 260º C split injection (7:1) on to a 25m J&W Ultra 2 column programmed at 60º (1 minute) -10º/minute -315º (5.5 minutes). The concentration of methadone was determined by comparing the peak area ratios of methadone to the internal standard against a standard 4 point calibration curve. Linearity was demonstrated up to 1.0 mg/L, with fractional volumes being used for samples exceeding linearity.

A discussion of case circumstances will include past pediatric methadone cases, ruled undetermined and accidental, covering the ranges of 0.07 mg/L-0.46 mg/L toxicity levels of this synthetic narcotic.

Methadone, Pediatric, Fatalities

K39 Postmortem Concentrations in a Suspected Nikethamide Death

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After attending this presentation, attendees will learn about nikethamide, its use and potential toxicity, as well as postmortem concentrations found in blood, urine, liver tissue, and brain tissue in a suspected nikethamide death case.

This presentation will impact the forensic community and/or humanity by primarily providing the forensic toxicology/pathology community with postmortem concentrations in various bodily fluids and tissues from a suspected death case for which no readily apparent levels have been previously reported.

The decedent in this case was a sailor on a transport ship traveling from South America to the United States. Shortly after leaving a South American port, the individual in this case was discovered dead in the ship's engine room. A search of the decedent's cabin did not reveal any evidence of drug use; however, an investigation of the crew disclosed that the decedent, along with a couple of friends, went ashore and returned to the ship shortly before it set sail. As part of the postmortem examination, bodily fluids and tissues were submitted for toxicological testing. These tests included assays for carbon monoxide, alcohols, common substances of abuse and therapeutic drugs. The findings of the screen tests on cardiac blood detected and identified nikethamide by gas chromatography/mass spectrometry (GC/MS). Further quantitative testing for nikethamide by gas chromatography with nitrogen-phosphorus detection found 32 mcg/mL in cardiac blood, 3.6 mcg/mL in urine, 22 mcg/g in liver and 2.6 mcg/g in brain tissue. Other than an incidental finding of caffeine no other findings of toxicological significance were detected.

Nikethamide is a central nervous system (CNS) stimulant that causes an increase in the respiratory rate through its direct action on the brain or by indirect action on the carotid chemoreceptor. Although it has no direct affect on the heart or the blood vessels, it can cause an increase in the heart rate and blood pressure. It has been used to treat respiratory and/or circulatory depression caused by central nervous system agents such as barbiturates, alcohol, opiates, etc., as well as cholinesterase inhibitors and carbon monoxide. Nikethamide has also been used in patients in shock, respiratory failure secondary to chronic obstructive respiratory disease, and cardiac decompensation and coronary occlusion. It is available as a powder and as a solution for oral and parenteral injection. The usual dose is 0.5 to 1 gram intravenously, intramuscularly or subcutaneously. It is well absorbed and metabolized partly to niacinamide then further metabolized to N-methylniacinamide.

Nikethamide has a narrow margin of safety. The CNS stimulant effects produced by nikethamide for respiratory therapy may lead to generalized seizures and potentially death. As a result, the use of nikethamide as well as other similar types of drugs termed analeptics is strongly discouraged. Not only is nikethamide discouraged from clinical use, it is banned by the World Anti-Doping Agency. Nikethamide is on the NCAA Banned Drugs list forbidding its use. It also appears on several lists of banned substances in horse racing.

With no available information on blood or serum concentrations associated with nikethamide therapy or toxicity, or information regarding a lethal dose, the determined values were compared to reported levels of similarly acting analeptic drugs such as doxapram and pentylenetetrazol. Average peak plasma concentrations following a therapeutic infusion of doxapram ranged from 2.6 to 4.1 mcg/mL, with signs of toxicity expected at levels exceeding 9 mcg/mL of doxapram plus its metabolite, 2-ketodoxapram. Peak plasma concentrations of pentylenetetrazol following therapeutic dosages were reported to range from 1.5 to 3.1 mcg/mL.

Nikethamide, Postmortem, Toxicology

K40 Postmortem Redistribution of Phenobarbital: A Rat Suicide Model

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After attending this presentation, attendees will retain the importance of postmortem redistribution of phenobarbital (barbiturate), will understand grossly how redistribution works in a cadaver, and will learn some of the forensically important insect species in south-eastern Greece.

In this rat suicide model, phenobarbital is being used. It belongs to a category of drugs of which postmortem redistribution has not been studied for a long time. This presentation will impact the forensic community and/or humanity by demonstrating how the postmortem redistribution of a drug with this kind of profile is important to be known by a forensic toxicologist or forensic pathologist, so as to be able to interpret the results of blood, body fluids, and solid tissue samples.

Drug concentrations found in toxicological analyses of postmortem tissue, body fluids, and blood samples can lead to erroneous conclusions without the proper interpretation by the forensic pathologist, resulting in liability claims, insurance denials, and significant emotional turmoil for all involved. Postmortem redistribution of a drug may be the basis for elevated or toxic drug concentrations after death.

Postmortem redistribution refers to the processes by which diffusion of drugs and other chemicals takes place after death, from the gastrointestinal tract and solid organs to blood and other body fluids and vice-versa. This phenomenon is well recognized, and was first reported 25 years ago. Since then a considerable effort has gone into elucidating the processes responsible. Consideration of the redistribution of drugs is important in a variety of situations. Cases of suspected poisoning (either homicidal or suicidal) or cases where the drug concentrations are in the threshold of toxicity, as in vehicle accidents, and also potential cases of euthanasia or medical negligence, may rely absolutely upon the validity of toxicological analyses of blood and tissue samples obtained postmortem.

In this study, a suicide simulation model, 54 wistar rats were separated in six groups. In each experiment, six rats were sacrificed by intraperitoneal infusion of 300mg of phenobarbital dissolved in double distilled water (ddH_2O), and three by neck dislocation (controls). Each group was then exposed in open air for a different period of time: 0, 4, 5, 6, 7 and 8 days. At the times indicated the bodies were collected, and various carrion tissues (liver, kidneys, lungs, heart, and bloody fluid) and scavenger insect larvae were taken away for further analyses. For the toxicological analyses of the specimens (bloody fluid, tissue and larvae extracts), a Cobas Integra 400 plus (Roche Diagnostics) was used. This automatic analyzer performs measurements for the quantitative determination of drug concentrations using fluorescence polarization immunoassay (FPIA). In addition, scavenger insects were collected from the experimental scene, preserved in Kahle's solution, and were grouped and identified. Analytical climatological data were recorded, i.e. temperature (T, °C), relative humidity (RH, %) and rainfall height (r, mm), on an hourly basis.

The results indicate that there is a strong time-dependent linear increase in the levels of phenobarbital in the bloody fluid, heart, and lungs. In liver and kidneys a similar increase is initially noticed, but after the fifth day it is followed by linear decrease.

In larvae the decrease commenced the sixth day. The insects collected belong to the orders of diptera, coleoptera, and hymenoptera. The dominant order was diptera, family Calliphoridae, species Lucilia sericata.

Consequently, it is obvious that pathophysiology of decomposition plays an important and determinative role in the barbiturates related deaths. Various causes of death due to phenobarbital intake (suicidal deaths, euthanasia, accidental overdose deaths and homicides) can easily be confused. Death investigation and forensic toxicology are not immune to misinterpretation, as a large degree of error can arouse from attempting to estimate antemortem drug concentrations based only on single postmortem measurements.

Phenobarbital, Suicide Model, Postmortem Redistribution

K41 Prevalence of Diltiazem in Cocaine-Positive Postmortem Cases in Maryland

Rebecca A. Jufer, PhD, Barry S. Levine, PhD, and David R. Fowler, MD, Office of the Chief Medical Examiner, 111 Penn Street, Baltimore, MD 21201*

After attending this presentation, attendees will learn about the prevalence of diltiazem in cocaine-positive postmortem cases in Maryland from 1995 through June 2006.

This presentation will impact the forensic community and/or humanity by providing information about the increasing prevalence of diltiazem as a cocaine adulterant.

Diltiazem is a benzothiazepine calcium channel blocker that is indicated for the treatment of hypertension, angina, and supraventricular arrhythmias. In therapeutic situations, diltiazem is typically administered in doses of 60-240 mg daily, producing therapeutic concentrations in the range of 0.1 to 0.3 mg/L. The plasma elimination half-life ranges from 2.8 to 9.2 hours. Adverse effects that have been reported with diltiazem use include weakness, edema, dizziness, nausea, and vomiting. Bradycardia, hypotension, and cardiac failure have been associated with diltiazem overdose.

In humans, diltiazem is extensively metabolized by O- and N-demethylation, deacetylation, N-oxide formation, and conjugation. Many conjugated metabolites are excreted in the urine, while less than 1% of a dose is excreted unchanged.

The Office of the Chief Medical Examiner of the State of Maryland has seen an increase in the frequency of diltiazem in cocaine positive postmortem cases in recent years. Cocaine positive cases received from January 1, 1995 through June 30, 2006 were reviewed to determine the prevalence of diltiazem in cocaine positive cases. Diltiazem and cocaine were identified in an alkaline drug screen, which involved an alkaline extraction of specimens followed by detection with gas chromatography /nitrogen-phosphorous detection and confirmation by gas chromatography / mass spectrometry. The results are summarized below.

Year	# COC positive cases	# COC and DILT positive cases	% DILT positive cases
1995	446	0	0.0%
1996	464	4	0.9%
1997	496	2	0.4%
1998	493	3	0.6%
1999	443	3	0.7%
2000	382	0	0.0%
2001	235	0	0.0%
2002	446	2	0.4%
2003	483	2	0.4%
2004	444	7	1.6%
2005	422	24	5.7%
2006- 1st half	256	45	17.6%

As seen in the above data, there has been a dramatic increase in the number of cocaine positive cases containing diltiazem in the past year. The Drug Enforcement Administration first reported cocaine seizures adulterated with diltiazem in 2004.[1] Since 2004, there have been several additional reports of diltiazem adulterated cocaine seizures in various areas of the country.[2-6] When quantitated, the diltiazem content of these seizures ranged from 8 to 20 %. These reports indicated that the reason for the selection of diltiazem as an adulterant is unknown.

The cardiotoxic effects of cocaine include hypertension, myocardial infarction, ventricular and supraventricular arrhythmias and tachycardia. As a calcium channel blocker, diltiazem could potentially offer some protection from the cardiac dysrhythmias induced by cocaine. One study examined the interaction between calcium channel blockers and cocaine in humans and found that pretreatment with diltiazem did not affect cocaine induced increases in blood pressure, heart rate, pupil size or subjective "high" ratings.[7]

Although the reason for adulteration of cocaine with diltiazem is unclear, the identification of diltiazem in a case may assist with identifying the source of the illicit cocaine. While there could potentially be an interaction between the two drugs, this trend is very recent in Maryland and the data are too limited to determine if the use of diltiazem as a cocaine adulterant has a role in cocaine-related death cases.

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Cocaine, Diltiazem, Postmortem

K42 General Unknown Screening of Drugs and Toxic Compounds in Human Samples Using a Hybrid Triple Quadrupole/Linear Ion Trap LC/MS/MS System

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After attending this presentation, attendees will learn about using LC/MS/MS for a general toxicology screen. Sample preparation is simplified versus other screening techniques. This general screening technique also has the capability to detect unexpected drugs and metabolites, as well as targeted analytes.

This presentation will impact the forensic community and/or humanity by demonstrating a relatively new and novel screening technique that is, in general, simple and faster compared to most techniques. It allows faster and more specific detection and identification of analytes in a screening assay.

General unknown screening (GUS) procedures in clinical or forensic toxicology are used to detect and identify the exogenous compounds present in human samples, whether expected or not. A comprehensive LC/MS/MS GUS method has been developed for drugs, toxic compounds and their respective metabolites in biological fluids

A simple, non-selective solid-phase extraction sample preparation was used. The mass spectrometer is operated in the Information Dependent Acquisition (IDA) mode, where ions are selected from a single MS ion-trap survey scan and the two most intense ions are submitted for MS/MS acquisition. The complete cycle time lasts approximately 1.36 s. A library of MS/MS spectra of parent compounds and metabolites has been built up and the MS/MS spectra acquired can be searched against the library for analyte identification and confirmation.

This method proved to be very efficient to identify unexpected compounds in biological samples (as far as they corresponded to library entries), as well as to give clues about the presence of metabolites owing to MS similarities with their respective parent compound. More than 1000 MS/MS spectra in the positive mode and 250 in the negative mode were entered in the library, together with compound name, developed chemical structure, CAS number, retention time, relative retention time and UV spectrum. Clinical cases will be presented where compounds not found by other screening or target techniques could be identified unambiguously.

A unique combination of the innovative operating modes offered by hybrid triple-quadrupole linear ion-trap mass spectrometers and new software features rendered it possible to develop a comprehensive and efficient method for the General Unknown Screening of drugs, toxic compounds, and metabolites in blood or urine.

Toxicology, LC/MS/MS, Drug Screening

K43 Determination of Alprazolam in Oral Fluid

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After attending this presentation, attendees will learn the disposition of alprazolam in oral fluid and understand the degree of sensitivity necessary for its detection.

This presentation will impact the forensic community and/or humanity by demonstrating how, as oral fluid gains acceptance as a suitable specimen type for roadside collection, procedures for the detection of low level benzodiazepines are very important.

Methods: Oral fluid is increasingly being studied as a suitable matrix for roadside collection and determination of driving under the influence of drugs. A low dosage, potent benzodiazepine with anxiolytic properties, alprazolam, was selected for this experiment, due to its potential contribution to impaired driving. Benzodiazepines have not been widely detected in oral fluid since the saliva:plasma (S:P) ratio is less than 0.5 for most of the drug class. The newer benzodiazepines are also given in low dosage regimens making their detection in oral fluid even more difficult.

Extraction: Calibrators were prepared in Quantisal™ transportation buffer at concentrations of 0.1, 0.2, 0.5, 1, 2 and 5 ng/mL of alprazolam. Deuterated (d5) alprazolam was added at a concentration of 5 ng/mL. Mixed mode (cation exchange:hydrophobic) solid phase extraction columns were conditioned with methanol (3 mL), deionized water (3 mL) and 0.1M phosphate buffer (pH 6.0; 2 mL). The specimens were loaded onto the column and allowed to run through. The columns were washed with deionized water (3 mL) and 0.1M phosphate buffer:acetonitrile (80:20, v,v: 2 mL). The columns were dried for 5 minutes, then hexane (1 mL) was added. The alprazolam was eluted with ethyl acetate:ammonium hydroxide (98:2 v,v; 2 mL) and

evaporated to dryness. BSTFA + TMCS (50 □L) was added and the samples were heated at 70ºC for 45 min.

Analysis: In order to achieve the sensitivity necessary for the detection of low level anxiolytic benzodiazepines in oral fluid, a two-dimensional gas chromatographic system was employed, with negative chemical ionization mass spectral detection. The system functioned optimally when the phases of the two gas chromatographic columns were as different as possible. The primary gas chromatographic column was a DB-35 MS column (30m x 0.25mm ID x 0.25 µM film thickness), the inlet pressure was 54.3 psi, and the average linear velocity was 81 cm/sec. The length of the restrictor column was calculated by software, and was dependant on the dimensions of the column and the pressure. The restrictor tubing was connected to the Deans switch and the other end was attached to a secondary detector.

In a Deans switch mode, the flow from the primary column plus a switching flow are passed onto the secondary column. The secondary column was a DB-1 stationary phase (15 m x 0.25 mm i.d. x 0.25 □m film thickness). The Deans Switch (Auxiliary Port #3) was programmed to operate at a pressure of 31.2 psi. It allowed all the flow from the primary column to vent through the flame ionization detector for 11.2 min. For 1.1 min the flow was then switched to allow the carrier gas to enter the secondary analytical column. At 12.3 min, the flow was returned to the secondary vent.

In order to "trap" the analyte using the cryo-focusing unit, the focuser was cooled from the oven temperature of 280ºC to 100ºC beginning at a run time of 10.5 min. The ramp rate for cooling was as high as it was possible to set the software and was set at 777ºC/ minute. It was held at 100ºC for 3 min, thereby allowing the alprazolam to trap in the cryofocuser. At a retention time of 13.5 min, the focuser was heated at a rate of 777ºC/minute to a final temperature of 280ºC.

Injection and Oven Parameters: The front inlet was operated in pulsed splitless mode at an initial temperature of 280ºC. The pressure was 54.3 psi and the pulse time was 1 minute. The purge flow was 20 mL/min and the purge time was 1 minute. The injection volume was 2 µL.

The oven was programmed from 190ºC for 1 min; ramped at 30ºC/min to 320ºC where it was held for 10.67 min.

Mass Spectrometer Parameters: The instrument was tuned in negative chemical ionization mode, using ammonia. The flow of the ammonia collision gas into the source was maintained between 8.0 x 10^{-5} and 1.0 x 10^{-4} Torr. The MS source was held at 150ºC, the quadrupole at 106ºC, the transfer line at 280ºC, and was operated at 800eV over tune. The MSD was operated in selected ion monitoring mode with four ions in a single group. Ions 313 and 315 were monitored for D_5-alprazolam; 308 and 310 for alprazolam with a dwell time of 50 ms for each ion. The retention time of alprazolam was 14.4 min. The method was linear over the range tested.

Results: The procedure was applied to specimens collected using the Quantisal™ oral fluid collection device, from a subject who was a prescription user of alprazolam. The profile of alprazolam detection over a time course of 16 hours after ingestion will be presented.

Summary: A method for the extraction and highly sensitive detection of alprazolam in oral fluid is described. The method was applied to oral fluid specimens taken from a prescription user of alprazolam.

Alprazolam, Driving Under the Influence of Drugs, Oral Fluid

K44 Stereoselective Determination of Methamphetamine From Urine Using Purge and Trap GC/MS

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After attending this presentation, attendees will be familiar with a stereoselective and sensitive methodology for the determination of methamphetamine in urine samples. Attendees will be familiar with this novel technique for separating methamphetamine isomers using PT-GC/MS. An indirect chiral separation using the optically pure chiral derivatizing reagent TPC is presented to the attendee. Attendees will understand the pre- concentration capabilities of dynamic headspace sampling and how pivotal it can be when analyzing biological fluids containing analytes at low concentrations. The attendee will be presented with analytical figures of merit for this technique and comparisons will be made with more traditional techniques such as SPE-GC/MS.

This presentation will impact the forensic community and/or humanity by providing the forensic community with a new analytical tool capable of identifying the individual isomers of methamphetamine in urine with minimal sample preparation. Such capabilities would not only assist forensic toxicologists, but would also provide a new technique capable of identifying enantiomeric ratios which provide pivotal information regarding the method and origin of clandestine methamphetamine synthesis.

Quantitative and stereoselective determination of methamphetamine from urine using purge and trap gas chromatography-mass spectrometry (PT-GC/MS) is described. Methamphetamine is an optically active sympathomimetic amine existing in two isomeric forms. The dextrorotatory [d-(+)] form of methamphetamine which is often prepared from ephedrine, induces central nervous system (CNS) stimulant effects and as a result is more widely abused than its legally available levorotatory [l-(-)] form. Although both enantiomers are considered controlled substances under United States regulations, there is still a need to develop enantioselective methodologies capable of distinguishing the illicitly manufactured d-isomer from the legally available l-isomer in various matrices. A wide variety of optically pure pre-column derivatizing agents have allowed for the enantioselective determination of many isomers in biological fluids using achiral chromatography. The method described here utilizes a rapid pre-column derivatization of the methamphetamine isomers using trifluoroacetylprolyl chloride (TPC), allowing subsequent separation of the diastereoisomers on an achiral GC column employing MS detection. In recent years, both direct and indirect chiral separations have utilized a wide variety of instrumentation including GC/MS, HPLC, CE, SFC, TLC, and CEC to successfully separate the isomers of many chiral drugs. Such methodologies are necessary due to the large number of drugs possessing chiral centers which are either used therapeutically or abused, and whose individual enantiomers induce varying degrees of therapeutic implications, side effects, or in the case of methamphetamine, CNS stimulation. New techniques capable of optically resolving these drugs on an analytical scale would allow analysts to further understand the pharmacokinetics associated with individual enantiomers of drugs known to undergo stereoselective disposition following administration. Methamphetamine is synthesized clandestinely with average purities ranging between 50-70%, and, although it is well documented that the d-isomer is responsible for the CNS stimulant effects, quantitative analysis of individual isomers can indicate the route and origin of synthesis. Although stereoselective

determination of methamphetamine from urine has been achieved prior to this study, significant sample cleanup and/or derivatization techniques have resulted in time consuming and challenging methodologies. Concentrations of methamphetamine in urine can vary significantly depending on the dose and whether or not the subject is a regular abuser. As a result there is often a need for sample extraction/pre-concentration from complex matrices. Solid-phase extraction (SPE) followed by pre-column derivatization has proven to be a successful preparative technique for the separation of methamphetamine isomers in urine using GC/MS; however there is a need for more convenient, time efficient techniques. The current methodology describes the stereoselective quantification of methamphetamine isomers in urine samples while reducing the degree of sample preparation. Rapid pre-column derivatization allowed for the subsequent extraction and pre-concentration of the diastereoisomers using dynamic headspace sampling followed by GC/MS.

Methamphetamine, Stereoselective, GC/MS

K45 Tissue Distribution of Drug Intoxication in Pediatric Fatalities

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After attending this presentation, attendees will have learned about the tissue distribution of lidocaine in a pediatric fatality as well as the tissue distribution of methadone in four pediatric fatalities.

This presentation will impact the forensic community and/or humanity by demonstrating the importance of obtaining multiple tissue samples for analysis in pediatric fatalities involving drugs as well as the need for co-operation between pathology staff and the toxicology laboratory.

Lidocaine is a local anesthetic. Case history and toxicological findings from one pediatric fatality due to lidocaine intoxication is presented. Methadone is an analgesic. Case histories and toxicological findings from four pediatric fatalities are presented.

In the first case, a two year-old black female complained about a sore in the mouth, was taken by her mother to a clinic. The subject was given a prescription for lidocaine and was found unresponsive two days later. The subject was transported to the hospital and expired on the following day. Lidocaine was detected in a basic drug screening and quantitated by Gas Chromatography, after solvent-solvent extraction with internal standard methodology. The presence of lidocaine was confirmed with full scan Gas Chromatography/Mass Spectrometry. The tissue distribution of lidocaine for the first case was as follows: blood, 2.52 mg/L; bile, 1.98 mg/L; liver, 0.76mg/kg; brain, 0.52 mg/kg; spleen, 7.20 mg/kg; and kidney, 2.68 mg/kg. The cause of death was bronchopneumonia due to lidocaine intoxication due to herpes stomatitis. The manner of death was listed as accident.

In the second case, an eighteen month-old black male drank a glass of orange juice with methadone on an end table and was found unresponsive later. The subject was hospitalized and died five days later. Methadone was detected in a basic drug screening and quantitated by Gas Chromatography, after solvent-solvent extraction with internal standard methodology. The presence of methadone was confirmed with full scan Gas Chromatography/Mass Spectrometry. The tissue distribution of methadone for the second case was as follows: blood, 0.10 mg/L; hospital blood (clotted, day two), 0.10 mg/kg; liver, 0.23 mg/kg; and brain, 0.23 mg/kg. The cause of death was methadone intoxication. The manner of death was listed as undetermined.

In the third case, a two year-old black male was found choking and gasping for air while in the bed. The subject was hospitalized and expired three days later. The tissue distribution of methadone for the third case was as follows: blood, negative; bile, 0.36 mg/L; and liver, 0.26 mg/kg. The cause of death was methadone intoxication. The manner of death was listed as undetermined.

In the fourth case, a five year-old black female began to choke and went into convulsions in the presence of her grandmother early morning. The subject died in the emergency room. On the day before, the subject might have drunk some of the orange juice with methadone, while riding with her mother and one of her mother's friends in her mother's car. The tissue distribution of methadone for the fourth case was as follows: blood, 0.64 mg/L; urine, 3.31 mg/L; bile, 2.18 mg/L; liver, 2.22 mg/kg; brain, 0.82 mg/kg; and spleen, 3.58 mg/kg. The cause of death was methadone intoxication, with parental neglect as a contributing factor. The manner of death was listed as undetermined.

In the fifth case, a fourteen year-old black male was playing in a football game for his high school and later on that night, was complaining to his parents of having a headache. Four days later, the subject was vomiting, so his parents told him to stay home from school. The subject was found unresponsive in bed when his father returned home from work. The tissue distribution of methadone for the fifth case was as follows: blood, 0.35 mg/L; liver, 2.70 mg/kg; spleen, 1.08 mg/kg; and kidney, 1.48 mg/kg. The cause of death was methadone intoxication. The manner of death was listed as accident.

In these pediatric fatalities, multiple tissue specimens were submitted to the toxicology laboratory when the pathologist requested testing. In the event, that a positive finding occurred, in one specimen from the case, the toxicology staff was then able to analyze multiple tissue specimens in order to provide a tissue distribution study. In pediatric fatalities, a positive finding of a drug usually implied that someone other than the deceased child/infant was involved with the administration of the drug in question. A tissue distribution study performed in this type of case will provide the toxicology findings as an unequivocal litigation package. Toxicologists are only able to do tissue distribution studies in cases such as these, with the full support of the pathology staff.

Lidocaine Tissue Distribution, Methadone Tissue Distribution, Pediatric Fatalities

K46 Sensitive Detection of Amphetamines and Other Basic Drugs Using Eosin Isothiocyanate

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After attending this presentation, attendees will understand the growing applications of microfluidic systems such as how they can be used to solve crimes as well as diagnosis health issues.

This presentation will impact the forensic community and/or humanity by demonstrating that microfluidic systems can perform extremely rapid analyses of compounds utilized in crimes such as Drug Facilitated Sexual Assaults and DUIs.

The application of microfluidic systems to toxicological screening and clinical diagnostics is growing rapidly. Rapid analysis of small molecules is essential in the detection of drugs for the prosecution of crimes such as drug-facilitated sexual assault and DUIs. Detection of biogenic amines for identifying health disorders and diseases is also of

interest. Therefore, these systems are of interest to both criminal investigators and medical personnel. Microfluidic systems utilize very small quantities of samples (µLs) and perform rapid separations (2 min. or less). Their small size makes them potentially portable for use at crime scenes. In addition microchips can be inexpensively, and the designs are simple to minimize user interaction. Quick analysis (preferably on-site) and high sensitivity are crucial to detection because some drugs can be metabolized and rapidly eliminated.

Although liquid phase drugs are traditionally detected by UV, its sensitivity is limited in microfluidic analysis by short path lengths (approximately 50 µm or less). Fluorescence is by far the most common detection method utilized by microfluidic systems due to its high sensitivity. However, most drugs are not naturally fluorescent so analysis must be derivatized. Biogenic amines and many drugs of abuse which are primary or secondary amines can easily be derivatized by amine reactive dyes.

Alnajjar, et al presented a method for the derivatization of opiates and their derivation with fluorescein isothiocyanate (FITC) and detection by CE-LIF with a 488nm argon-ion laser.[1] The method presented is the detection of several phenethylamines, primary and secondary, by microchip CE-LIF. The drugs are derivatized by eosin isothiocyanate (EITC) and detected on a Micralyne microfluidic Tool Kit (µTK) equipped with a 532nm frequency-doubled laser. Tertiary amines can also be derivatized following a demethylation procedure.[2]

One of the advantages of microfluidic systems is their potential to perform simultaneous sample preparation, separation, and detection. This growing trend in microfluidics to create micro total analysis systems (µTAS) greatly improves the overall analysis speed. It has been shown that several phenethylamines[3] and biogenic amines[4] can be fluorescently derivatized in a few seconds making on-chip reactions feasible. A method for performing rapid derivatization of phenethylamines by EITC on-chip is proposed. Several chip layouts were tested to promote mixing of reagents, and very narrow reaction chambers improved mixing. The reactions were optimized by placing the mixed reagents in the dark at room temperature for 48 hours and measuring the product yield relative to an internal standard every few hours. Although it took about 24 hours for the reaction to go to completion, products formed at a detectable level in less than 10 minutes.

Derivatized amphetamine, methamphetamine, and ephedrine could be separated by CE with baseline resolution using a basic buffer, pH = 9.8, containing cyclodextrins and a separation voltage of 10 kV in a 40 cm long capillary. In addition microchip separation could be simulated on a traditional CE by injecting the sample on the short end of the capillary. The combination of the rapid microchip separation and the on-line sample preparation resulted in a µTAS which could perform a screening test in a matter of minutes.

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DFSA, Microfluidic, Fluorescence

K47 Validation of a Headspace-Gas Chromatography Method for the Analysis of Gamma-Hydroxybutyrate and Analogs

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After attending this presentation, attendees will have learned about the validation of a quantitative technique for the simultaneous analysis of GHB and GHV in beverages using an instrument common to toxicological analyses.

This presentation will impact the forensic community and/or humanity by demonstrating a quantitative technique for the detection of GHB and GHV in beverages using a common toxicological analysis.

Previous work from this laboratory has described validated headspace-gas chromatography-flame ionization (HS-GC-FID) and gas chromatography-mass spectrometry (GC-MS) method for screening and identifying gamma-hydroxybutyrate (GHB) and its lactone (gamma-butyrolactone, GBL) in biological fluids. An advantage of this approach is the use of the same analytical system as blood alcohol measurements; making this method readily available to toxicology laboratories. No sample preparation is required and the use of internal standards facilitates reliable quantitation.

Extensions of this method to other sample matrices and other target compounds will be presented. An analog of GHB, the five carbon gamma-hydroxyvalerate (GHV) and corresponding lactone (gamma-valerolactone GVL) have recently emerged as a predator drug threat. These analogs are characterized by larger dose requirements and thus greater potential for toxicity. Because of their chemical similarity to GHB/GBL, they are also amenable to this method. Results and method validation for these compounds will be discussed and compared to traditional GC-MS analyses. In addition, analysis of typical precursors and by-products will be addressed.

Headspace methods are also useful for physical evidence such as adulterated beverages. Given discrimination based on volatility and solubility, the headspace method effectively removes much of the interfering matrix while affording quantitative transfer of analytes to the analytical system. Results from the analysis of various beverages such as wine, beer, soda, and mixed drinks will be described with recoveries and interferents.

Headspace, GHB, GHV

K48 Rapid Analysis of THC and Metabolites Using Disposable Pipette Extraction

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After attending this presentation, attendees will understand a new and improved method for analyzing THC and metabolites in whole blood.

This presentation will impact the forensic community and/or humanity by assisting the forensic community to implement a faster and improved method for performing THC analysis.

A rapid extraction method for THC and metabolites has been developed using disposable pipette extraction (DPX). Although DPX has been previously introduced for this extraction methodology, improvements in the design of the DPX tips and changes in sorbent material have permitted higher recoveries and more reproducible data. Also, the extracts are much cleaner and negligible interferences were noted from actual case samples. Furthermore, the new method permits the simultaneous analysis of THC, OH-THC and COOH-THC in a single extract and thus single chemical derivatization.

The method involved extracting 1 mL of whole blood with 1.5 mL acetonitrile. After mixing, the supernatant was decanted into a clean labeled test tube and 2.5 mL of 0.1M HCl added. The mixture was drawn into the DPX tip using an attached 10 mL syringe device and mixed with the sorbent by drawing in air. After 30 seconds, the sample solution was dispensed back into the tube (or waste). Subsequently, 0.5 mL of methanol was drawn into the DPX tip and mixed as a wash step. For elution, 0.5 mL of 5:1 hexanes-ethyl acetate was drawn into the DPX tip, mixed with the sorbent by drawing in air, and after 10 seconds the eluent was dispensed directly into the corresponding labeled GC vial. The elution step was repeated with an additional 0.5 mL of 5:1 hexanes-ethyl acetate. The DPX extraction time, following the protein precipitation with acetonitrile, took approximately 3 minutes, and 12 samples could be processed simultaneously using 12 syringe devices.

Using BSTFA for derivatization, 3 ions (1 target and 2 qualifier ions) could be monitored for THC free from apparent interferences. GC oven temperature was held for about 5 minutes at 220 C to improve the chromatography and increased the resolution of interferences with THC. Detection limits were less than 0.5 ng/mL for THC and OH-THC and approximately 1ng/mL for COOH-THC.

THC, Disposable Pipette Extraction, Solid-Phase Extraction

K49 Evaluation of the Lin-Zhi International Opiate Enzyme Immunoassay for the Detection of Opiates in Urine

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After attending this presentation, attendees will learn the performance characteristics of the Lin-Zhi Opiate Assay, as they apply to urine samples and the availability of an alternate vendor for an opiate EIA method.

This presentation will impact the forensic community and/or humanity by reviewing current methods for opiate screening, with the characteristics of an alternate vendor to those currently available. At a minimum, expand the current available information on how robust current opiate methodologies are.

The Opiate Enzyme Immunoassay [OEI] (Lin-Zhi International, Inc., Sunnyvale, CA) for the detection of opiates in urine. The Lin-Zhi assay is based on competitive antibody binding between opiates in urine and glucose-6-phospahate dehydrogenase labeled morphine. The assay is calibrated with morphine.

The Lin-Zhi OEI was evaluated by testing 1212 urine specimens. All specimens were tested on an ADVIA 1200 Chemistry System auto-analyzer (Bayer Health Care, Diagnostics Division, Tarrytown, NY) with calibrators containing 0, and 300 ng/mL (cut-off calibrator) of morphine. Controls containing 0 ng/mL of morphine, 225 ng/mL (negative control) and 375 ng/mL (positive control) of the cut-off calibrator (Bio-Rad Laboratories, Irvine, CA) were analyzed with each batch of samples. All urines were then analyzed by a GC/MS for opiates at a cut-off concentration of 100 ng/mL.

Approximately, 58 % (711) of the 1212 specimens screened positive by the Lin-Zhi assay. GC/MS confirmed the presence of an opiate at >100 ng/mL, in 692 of the 711 specimens, indicating 19 false positive results. However, 88 specimens screening negative were found to contain an opiate above the GC/MS cut-off of 100 ng/mL (78 oxycodone and/or oxymorphone). Opiate concentrations in the specimens as determined by GC/MS, ranged from < 100 - >50,000 ng/mL. From the presented study, the sensitivity of the Lin-Zhi OEI was 0.887 and the selectivity 0.956. Testing at 1,000 mg/mL of other drugs of abuse or their metabolites such amphetamine, benzodiazepines, benzoylecgonine, morphine and phencyclidine, the Lin-Zhi assay demonstrated no cross reactivity. The within-run precision of the Lin-Zhi assay was determined by the absorbance rates of the negative and positive controls was CV£ 5% (n=8); while the between-run precision of the controls was CV=<5% (n=11). The assay was linear from 25% to 150% of cut-off concentration. The Lin-Zhi CBI provides a precise, reliable method for the detection of opiates in urine specimens.

Enzyme Immunoassay, Opiates, Urine Drug Testing

K50 Confirmatory Analysis of Ethylglucuronide and Ethylsulphate in Urine by LC/MS/MS According to Forensic Guidelines

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After attending this presentation, attendees will understand the use of LC/MS/MS technology to analyze alcohol metabolites.

This presentation will impact the forensic community and/or humanity by demonstrating a new LC/MS/MS based and validated method for the analysis of Ethylglucuronide and Ethylsulphate for forensic toxicology and workplace testing laboratories.

Ethylglucuronide (EtG) and Ethylsulphate (EtS) are stable Phase II metabolites of ethanol which can be detected in urine samples several days after elimination of ethanol. Determination in urine is mainly performed by LC/MS, LC/MS/MS, or by GC/MS. For the mass spectrometric identification and detection of controlled substances in sensitive fields such as forensic toxicology, workplace drug testing, doping analysis, and veterinary organic residue control, official guidelines have been released requiring a chromatographic separation and a minimum of two mass spectrometric transitions of detected analytes.

Therefore, an LC/MS/MS method was developed to detect the following transitions: deprotonated molecule of EtG [M-H]- to product ions m/z 75, 85, 159 and EtS [M-H]- to m/z 80, 97. Isotopically labeled internal standards were used to evaluate ion suppression effects. Simple dilution with water containing 0.1% formic acid followed by

centrifugation was found to be sufficient to prepare urine samples. HPLC separation was performed on a RP column using a gradient of water, acetonitrile, and formic acid. Post-column addition of acetonitrile was used to enhance sensitivity.

The method was validated regarding forensic guidelines. Urine samples were collected and analyzed after drinking experiments of volunteers. EtG and EtS were detected in these samples. Time plots are used to study the kinetics of metabolism of ethanol.

Ethylglucuronide, LC/MS/MS, Workplace Testing

K51 Driving Under the Influence of Drugs (DUID) Testing Protocol in the Commonwealth of Virginia

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After attending this presentation of the review of the driving under the influence of drugs protocol in Virginia, attendees will learn the system and see the stop analysis limits used in the state, which is related to the trial testimony.

This presentation will impact the forensic community and/or humanity by aiding toxicologists in reviewing testing protocol.

If law enforcement stops any driver suspected of DUID in the Commonwealth of Virginia, that driver is typically first administered a breath test for ethanol. If the ethanol result is too low to explain the police officer's observations, or if the suspect is unable to perform the breath test, the suspect is taken to a medical facility where two tubes of blood are collected. The blood samples are controlled under chain-of-custody until they arrive at the Department of Forensic Science Central Laboratory, usually via mail. One of the blood specimens is stored under chain-of-custody, and the other undergoes a graded tier testing scheme, developed to save resources and time and still assure the appropriate DUID analysis (i.e., full-spectrum drug analysis is not performed on each submission). The testing protocol will occur in at least one but up to five phases, as outlined below, and is referred to as:

Level I Testing For Ethanol
Level II Screen
Level II Confirmation
Level III Screen
Level III Confirmation

All specimens receive Level I Testing for ethanol. When ethanol is ³ 0.09% no testing beyond Level I is required, unless circumstances dictate that further testing be performed (e.g., involuntary manslaughter). When ethanol is < 0.09%, proceed to Level II Screening which includes immunoassay for barbiturates, cocaine/benzoylecgonine, benzodiazepines, cannabinoids, phencyclidine, and opiates. Any presumptive positive Level II Screen will automatically require a Level II confirmation and quantitation. If any value reaches the "Stop Analysis Limit" testing is concluded. Some examples of the stop analysis limits for Level II drugs are butalbital 10 mg/L, alprazolam .06 mg/L, tetrahydrocannabinol 0.002 mg/L and codeine 0.5 mg/L. Negative results in Level II require Level III Screening, which includes a screen for alkaline extractable drugs. Any presumptive positive Level III Screen will automatically require a Level III confirmation/quantitation by gas chromatography/mass spectrometry.

The tier system was designed so that once a drug, or group of drugs, was found at a concentration high enough for probable conviction of driving under the influence of drugs or alcohol, no other testing was done to detect other drugs. Thus, there is a potential for all drugs other than ethanol to be under represented. This tier system presents a potential retrospective problem if there is a necessity to determine "total drug usage by drivers" because the number of drugs found is clearly less than the "true or actual" number of drugs that were present in an individual at the time of the traffic stop. Underestimation is not a problem for ethanol because all blood samples were tested for ethanol. Using the tier system testing protocol causes Level II and Level III drugs to be under represented, but due to the design of the tier system, Level III drugs will be more severely under represented than the Level II drugs. Nevertheless, the tier system has proven quite successful in Virginia with regard to driver impaired convictions and enables more efficient utilization of resources and personnel in the Department of Forensic Science. Reporting limits and stop analysis limits are enumerated in this report.

DUID, Testing Protocol, Statistics Underestimation

K52 Methamphetamine and Phentermine in DUID Cases

Ashraf Mozayani, PhD, PharmD, Terry Danielson, PhD, and Luis B. Sanchez, MD, Harris County Medical Examiner Office, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will learn of some of the drugs frequently seen in combination with methamphetamine and of the effects on drivers impaired by such combinations. Phentermine is similar to methamphetamine, but is less potent as a sympathomimetic.

This presentation will impact the forensic community and/or humanity by demonstrating that a complete toxicological assessment is essential for the correct classification of the methamphetamine impaired driver.

This presentation will describe several Driving Under the Influence of Drugs (DUID) cases involving the sympathomimetic amines, methamphetamine, and phentermine. Methamphetamine produces a multi-phasic response with an initial excitation that is replaced, as drug levels decline, by feelings of drug craving, agitation, fatigue, and hypersomnolence. Users often co-administer alcohol, or other substances, such as anxiolytics, in attempts to "soften the crash."

By attending this presentation, attendees will learn of some of the drugs frequently seen in combination with methamphetamine and of the effects on drivers impaired by such combinations. Phentermine is similar to methamphetamine, but is less potent as a sympathomimetic amine. It is prescribed as an appetite suppressant and can be readily procured, even over the internet. It is not commonly associated with driver impairment and reports of detrimental effects on the operation of a motor vehicle are scant.

Specimens collected during DUID investigations were tested for ethanol by headspace gas chromatographic analysis and for amphetamine, methamphetamine, benzoylecgonine, marijuana, opiates, benzodiazepine, methadone, phencyclidine, and barbiturates by an Elisa technique. Drugs, including methamphetamine and phentermine, were confirmed and quantified by gas chromatography / mass spectrometry after derivitization with heptafluorbutyric anhydride.

Of nine recent methamphetamine DUID cases, eight were found to be positive for additional drugs. Only one case was positive for methamphetamine alone while two contained five additional drugs. Alprazolam or marijuana was each present in five cases, cocaine in four, methylenedioxymethamphetamine in three and ethanol in three. Eight of these drivers either crashed their vehicle or were unable to maintain a single lane of traffic. On-scene assessments by Drug Recognition Experts correctly identified central nervous stimulants in two cases; methamphetamine, cocaine, alprazolam in one case and ethanol cocaine and methamphetamine in the other. Four cases were classified as involving central nervous system depressant(s)

In another DUID case, phentermine in blood (0.25 mg/L) was combined with ethanol (0.13 g/dL), citalopram (0.1 mg/L) and zolpidem

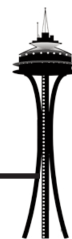
(0.09 mg/L). The accused drove over a curb and onto a sidewalk and either failed, or was unable to perform, standard roadside sobriety tests. The driver later admitted to use of zolpidem to enhance the effects of ethanol. Phentermine use was acknowledged only as a diet aid. Anecdotal evidence from other cases suggests, however, that phentermine may modify, or intensify, the effects of ethanol.

This study indicates that methamphetamine-users very often abuse multiple drugs and that such combinations can result a serious impairment. Furthermore these combinations often complicate on-site classifications, even by a skilled assessor.

Phentermine is a seldom recognized agent that may modify, or enhance, responses to ethanol. Little data is available to make an assessment of its effects on driving, either alone or in combination. However, it is available, even over the internet and opportunities for abuse do exist. Phentermine may be worthy of greater scrutiny in DUID cases. Literature data indicates that levels in blood as high as 0.5 mg/L might be attained after repeated dosing.

Most importantly this presentation indicates that a complete toxicological assessment is essential for the correct classification of the methamphetamine impaired driver.

Phentermine, Methamphetamine, DUID



K1 Clinical vs. Forensic Toxicology - A Comparison of Methods for Case Evaluation

David M. Benjamin, PhD, 77 Florence Street, Apartment 107, Chestnut Hill, MA 02467; and Robert H. Powers, PhD*, State of Connecticut Toxicology Laboratory, 10 Clinton Street, 4th Floor, Hartford, CT 06106*

After attending this presentation, attendees will be able to identify the similarities and differences between the practice of clinical and forensic toxicology. Toxicologists will be able to identify the limitations involved in relying on pooled or random mean blood levels and ranges.

This presentation will impact the forensic community and/or humanity by assisting forensic experts in identifying the limitations of relying on pooled, random blood level concentrations published in the professional literature. The need to standardize the units of concentration will be presented, and acceptable practices recommended.

Clinical and forensic toxicology often share the objective of trying to determine the toxic agent in patients or subjects. However, while medical and clinical toxicologists are chiefly involved directly with patient care, forensic toxicologists often deal with retrospective data involving a past event or death. In contrast to clinicians, forensic toxicologists are frequently called upon to help the courts resolve disputes in which drug toxicity has been a factor. Because forensic toxicologists often deal with cases years after the actual event, they lack the advantage of having been present at the time of the patient's treatment, and frequently lack critical laboratory test results which were not ordered by a clinician whose priorities were to try to save the patient, not determine a cause and manner of intoxication and/or death. Despite sharing a common body of knowledge, clinical and forensic toxicologists generally see cases involving a different spectrum of drugs, drug combinations, and dosages. The suicidal patient who intentionally overdoses on massive doses of his/her prescription medications differs significantly from the drug addict who inadvertently overdoses on "street drugs" taken to become euphoric or prevent withdrawal. Both of these scenarios differ from the patient presenting to the ER with unexpected side effects from a new medication, or inadvertent drug/toxin exposure. Clearly, any case can "convert" from a strictly medical or clinical exercise to a post-mortem forensic case, based on the outcome.

In addition to the differences between clinical and forensic toxicology described above, both specialties rely on different batteries of laboratory tests and literature sources generally utilized in the practice of their professions. Patient-centered toxicologists treat the signs of drug overdoses and poisonings, relying on non-specific screening tests as guides while employing life-saving interventions to support the patient's respiration, blood pressure and cardiac function. Sensitive, quantitative GC/MS results cannot generally be obtained within a rapid enough turn-around time to assist the clinician before the patient expires or recovers and specific information beyond the identification of a suspected toxidrome may be of limited use to the clinician. Forensic toxicologists generally employ sophisticated methodologies which can determine the presence of suspected drugs down to the nanogram level. While clinicians rely heavily on the a prescription drug's product labeling, and textbooks such as Goodman and Gilman's The Pharmacological Basis of Therapeutics and Ellenhorn's Medical Toxicology for recommendations on treatment, forensic toxicologists frequently cite blood level data from Baselt's Disposition of Toxic Drugs and Chemicals in Man. This commonly employed forensic reference has a more chemical and quantitative orientation, and is designed not to aid in the treatment of toxic patients, but to present a compendium of analytical data from drug cases involving reports of toxic or lethal outcomes. Cases reported in Baselt's book report drug blood levels of unspecified source and timing, and often combine the results of many incidents which may involve polypharmacy. Interpretation of the data may be further confounded by a lack of information regarding the time of drug ingestion, co-ingestions, and the presence of other drugs or factors affecting metabolism (e.g., induction, inhibition, or pharmacogenetic expression of the CYP 450 enzymes.) Moreover, interpretation of the data from "Baselt" may be further complicated by post-mortem redistribution, and a lack of specifics regarding the site from which the blood sample was obtained (e.g., right atrial vs. left ventricular vs. peripheral venous blood), the type of anticoagulant that was used (if any) and the presence or absence of NaF or other preservatives to retard or eliminate post-mortem production of ethanol or bacterial degradation of drugs.

This presentation will review differences between the clinical and forensic toxicology literature regarding certain drugs that frequently are encountered by both groups of professionals. These drugs include: ethanol, alprazolam, tricyclic antidepressants, local anesthetics, and morphine. Blood level data and the use of the appropriate units of measure from respective literature sources will be compared and contrasted in an effort to highlight the similarities and differences between the populations of patients (subjects) from which the samples were drawn, and recommend preferred practices. The potential for errors in interpretation will be presented in relation to the use of unreliable techniques (e.g., the use of single blood level values and "Volume of Distribution" to calculate the ingested dose). The risks associated with an uncritical reliance on reports of "mean blood concentrations" and ranges for toxicity and fatality published in "Baselt" will also be presented.

Interpretation Errors, Reliability, Postmortem Distribution

K2 Application of Laboratory Information Management Solution Software System Supporting Forensic Toxicology Operations

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After attending this presentation, attendees will learn the application of the Laboratory Information Management Solution (LIMS) software in a forensic toxicology operation.

This presentation will impact the forensic community and/or humanity by providing examples and detailed information on the toxicology LIMS software system, illustrating that the system can be used in laboratories to maximize their operation and services. Use of this type of software system can effectively improve multiple aspects of laboratory performance required by current scientific and legal standards.

The Federal Aviation Administration's Civil Aerospace Medical Institute (CAMI) toxicologically evaluates postmortem biological samples collected from victims involved in transportation accidents. Such biosamples are analyzed for the presence of primary combustion gases (carbon monoxide and hydrogen cyanide), alcohol/volatiles, and drugs. During the entire evaluation process, beginning with receiving samples through dispatching toxicology reports, there is a critical need to ensure the quality and integrity of the chain-of-custody, demographic, accessioning, and analytical data/records. Additionally, retrieving case-related information is frequently desired in an expedited manner. Therefore, an effective quality assurance/quality control (QA/QC) program is an absolute

necessity. Information pertaining to these case-related components could effectively be achieved using a suitable software system.

Based on the need for this approach, the CAMI Laboratory has been using the LIMS software since 1997. Initially, this system was tailored to fulfill the unique needs of the Laboratory. However, since the inception of this software system, it has been going through continuous developmental improvements and has become a dynamic forensic toxicology application, designed with input from the biologists, chemists, and toxicologists. Characteristics of this software system are described herein.

This software system has the components to allow laboratories to meet the requirements necessary to conform to the accreditation standards of the College of American Pathologists, the American Board of Forensic Toxicology, and any similar agencies. The basic components are oriented toward a forensic laboratory, covering sample receiving, report generating, record maintaining, QA/QC monitoring, and associated rapid information retrieving.

Specific features of the software include the ability to reliably track the chain-of-custody and acceptance of unlimited specimens per case, utilizing barcode labels created for all specimen vials. Information pertaining to the types and stability of blind QA/QC samples can be created, thereby allowing the accumulated specimen history to be easily tracked. Samples of analytical batches may be re-accessioned for additional analysis. The final case and batch information is locked from changes when completed. A case status snapshot feature shows the progress of a case. Multi-level security prevents analysts from being aware of the cases they are analyzing. If required, additional process-specific modules can be easily incorporated into the system. For example, incident reporting and Freedom of Information Act (FOIA) request processing modules have been easily added.

A case-edit-history view is available for upper-level management. This feature displays case or batch edits including date, time, and user. Management can also view system login history. Requests for case information under the FOIA can be easily tracked. Analytical and statistical report capabilities include information pertaining to QA/QC, internal and external specimen chain-of-custody, case status, and other specialized aspects of a case. Analytical reports can be easily generated through the batch-based case results with an option to include any notes that might enhance the interpretation of the analytical findings by report receivers. Laboratory incidents, along with their evaluations/resolutions and cost, are documented with a Lab Incident Report methodology. An archive feature stores historical data in a separate location, while preserving easy access to needed information. Data can be exported to a Microsoft® Excel worksheet, and report information to a Microsoft® Word document. The dynamic character of the LIMS makes it user-friendly and suitable for rapidly extracting information necessary for research. In essence, this software system is an effective tool to optimize the operation of a laboratory, covering its entire operational spectrum.

Forensic Sciences, Toxicology LIMS Software, Aviation Accident Investigation

K3 A Validated PCI GC/MS Method for the Quantification of Amphetamine, Opiates, Cocaine and Metabolites in Human Postmortem Brain

Ross H. Lowe, PhD, Allan J. Barnes, BS, and Marilyn A. Huestis, PhD, Chemistry and Drug Metabolism Section, National Institute on Drug Abuse, 5500 Nathan Shock Drive, Baltimore, MD 21224*

After attending this presentation, attendees will learn about a sensitive and specific method for the simultaneous detection and quantification of amphetamine, opiates, cocaine, and cocaine metabolites. The presentation will allow an attendee to evaluate the performance characteristics and implement the assay in their laboratory.

This presentation will impact the forensic community and/or humanity by demonstrating an assay which provides reproducible recovery and quantification of amphetamine, morphine, codeine, 6-acetylmorphine, cocaine, benzoylecgonine, ecgonine methyl ester, ecgonine ethyl ester, cocaethylene, and anhydroecgonine methyl ester in human brain tissue. The assay has application in forensic and postmortem toxicology laboratories.

Determination of drug concentrations in human brain has applications in forensic and postmortem toxicology and in biological studies of cellular responses to drug exposure. Direct measurement of drug and metabolite concentrations in discrete brain regions also is used to study mechanisms of drug action, regional distribution, and preferential accumulation of drugs. Most quantification methods have focused on a single class of drugs, such as cocaine, amphetamines, or opiates. The objective of this study was to develop and validate a reliable extraction and quantification method for multiple classes of drugs in brain tissue.

The method employs ultrasonic homogenization of brain tissue in pH 4.0 sodium acetate buffer and solid phase extraction (SPE) utilizing copolymeric octyl/benzyl sulfonic acid extraction columns. Extracts were concentrated and derivatized with *N*-methyl-*N*-(tert-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) and *N*,O-*bis*(trimethyl) trifluoroacetamide (BSTFA). GC/MS analyses were performed with an Agilent 6890 gas chromatograph interfaced with a 5973 mass-selective detector. Analyte separation was achieved on an HP-1MS capillary column (30 m x 0.32 mm i.d., 0.25 μm film thickness) with helium carrier gas. Initial column temperature of 70°C was held for 1.00 min, increased to 175°C at 30°/min, ramped to 250°C at 23°/min and increased to a final temperature of 310°C at 18°/min that was held for 5.00 min. The MS was operated in PCI mode with methane reactant gas. Target and qualifier ions acquired for each analyte and deuterated internal standard were: amphetamine 158, 250; amphetamine-*d10* 162, 245; ecgonine methyl ester 314, 256; ecgonine methyl ester-*d3* 317, 259; anhydroecgonine methyl ester 182, 210; ecgonine ethyl ester 328, 196; cocaine 304, 182; cocaine-*d3* 307, 185; cocaethylene 318, 196; cocaethylene-*d3* 321, 199; codeine 282, 356; codeine-*d3* 285, 359; benzoylecgonine 404, 282; benzoylecgonine-*d3* 407, 285; morphine 456, 382; morphine-*d3* 459, 385; 6-acetylmorphine 382, 470; and 6-acetylmorphine-*d3* 385, 473, respectively.

Developing a validated method for simultaneous quantification of multiple drug analytes in human brain required optimization of numerous factors. First, a technique for successful tissue disruption coupled with an efficient extraction methodology was required. This was addressed by brief ultrasonic homogenization of 0.10 g of tissue in pH 4.0 sodium acetate buffer followed by centrifugation. SPE was rapid and reproducible with suitable recoveries, and required small volumes of organic solvents. Second, the need to quantify multiple analytes at low concentrations required reliable chromatographic separation of analytes and a suitably specific and sensitive detection method. A third objective was to utilize instrumentation readily available in most research and forensic toxicology laboratories, which was met by a bench-top GC/MS operated in PCI mode. Each analyte was adequately resolved from other analytes or from tested interferents with the chromatographic parameters described. Positive chemical ionization GC/MS in SIM mode provided sensitive and specific quantification.

Linearity, carryover, limits of detection and quantification, selectivity, extraction efficiency, precision and accuracy were investigated to evaluate method integrity. The limits of detection and limits of quantification for all analytes were 50 pg/mg of brain. Calibration curves were linear to 1000 pg/mg for anhydroecgonine methyl ester and 6-acetylmorphine, and to 2000 pg/mg for all other analytes. Precision and accuracy were evaluated over the linear range with four QC materials at target concentrations of 120, 240, 480, and 1600 pg/mg. Accurate quantification and precision is achieved over the linear dynamic range of the assay with accuracy ranging from 89.5% to 113.7%, and inter-assay precision, as percent relative standard deviation, ranging from 3.0 to 16.6%.

The method provided adequate and reproducible recovery of amphetamine, morphine, codeine, 6-acetylmorphine, cocaine, benzoylecgonine, ecgonine methyl ester, ecgonine ethyl ester, cocaethylene, and anhydroecgonine methyl ester from human brain tissue. The assay was developed to identify and quantify drugs in human postmortem brain tissue and to identify drug users and validate controls for microarray analysis of the transcriptional neurobiology of drug abuse.

Drugs of Abuse, GC/MS, Brain

K4 Validity of the Cozart Rapiscan Test for Drug of Abuse Screening in Hair by GC/MS Confirmation

Roberto Gagliano-Candela, PhD, Lucia Aventaggiato, Anna Pia Colucci, PhD, and Giuseppe Strisciullo, University of Bari, Dipartimento Medicine Interna Medicina Pubblica, Policlinico, Piazza G. Cesare n.11, Bari, 70124, Italy*

After attending this presentation, attendees will understand the analysis of opiates, cocaine and cannabinoids in hair by Cozart Rapiscan oral fluid Test® and GC/MS confirmation.

This presentation will impact the forensic community and/or humanity by providing information on the testing of drugs of abuse in hair.

Goal: This project was carried out to evaluate the performance characteristic of the immunoassay Cozart Rapiscan oral fluid Test® for drugs of abuse screening in hair extracts.

Methods: Hair samples (70) collected from dope addicts and drug-involved deaths in 2004 were selected from routine analysis samples at the Forensic Toxicology Laboratory, Bari University. The method involves decontamination in 1% sodium dodecyl sulfate, distilled water, and methanol, pulverization in a ball mill, overnight extraction in methanol at 60°C. The methanol extract was then blown until dry under nitrogen and reconstituted in 140 mcL of Cozart buffer for immunoassay analysis. Both positive and negative samples were confirmed by gas chromatography-mass spectrometry (GC/MS/EI) operating in selected ion monitoring mode. Before extraction, deuterated internal standards were added to hair specimens. For opiates and cocaine metabolite analysis, BSTFA/TMCS 1% silylation was used. The 72 positive results were confirmed by GC-MS analysis.

Sensitivity and specificity: The number of true positives, false negatives, false positives and true negatives was determined by comparison of the Cozart results to GC-MS as the reference method. Sensitivity, the true-positive rate, was calculated from the totality of true positives and false negatives as TP/(TP + FN). Specificity was calculated as TN/(TN + FP).

Results: The confirmation in GC/MS determined 39 true positives for opiates, 18 for cocaine, and 15 for delta-9-THC versus 72 total positive results. True negatives were 11 for opiates, 32 for cocaine and 35 for delta-9-THC. False negatives were 1 for cocaine and 3 for delta-9-THC. No false positive results were obtained.

The Cozart Test for opiates in hair, using a cut-off of 0.2 ng/mg with a 50-mg hair sample, had a sensitivity of 100% and specificity of 100%. The Rapiscan Test for cocaine in hair, using a cut-off of 0.5 ng/mg with a 50-mg hair sample, had a sensitivity of 94.7% and specificity of 100%. The Cozart Test for delta-9-THC in hair, using a cut-off of 0.5 ng/mg with a 50-mg hair sample, had a sensitivity of 83.3% and specificity of 100%.

Conclusions: The Cozart Rapiscan oral fluid Test® revealed good sensitivity and maximum specificity, proving to be a valid method of screening. To ensure the legal validity, confirmation analysis with chromatographic techniques (GC/MS or HPLC/MS) is required.

Hair Analysis, Drug Screening Analysis, Cozart Rapiscan

K5 Identification of Fentanyl in Urine From Drug Abuse Cases Using a Direct Multistage Mass Spectrometry Method

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After attending this presentation, attendees will add to their knowledge the use of ion trap and similar mass spectrometers for the identification of drugs of abuse in urine. The direct-injection, multistage mass spectrometric methods are faster and often more specific than traditional mass spectrometric identification methods.

Because the method involves direct injection into a mass spectrometer and does not require a chromatographic step, this presentation will impact the forensic community and/or humanity by providing considerable savings of time and costs compared to the application of mass spectrometric methods for the identification of fentanyl currently in the literature.

Multistage mass spectrometric analysis has become a powerful tool for quantitative confirmatory analysis of chemicals and drugs of abuse and has begun to spread in the field of forensic toxicology. In this presentation, the identification of fentanyl from six fentanyl positive cases provided by the Office of the Chief Medical Examiner of West Virginia is discussed.

The application of multistage MS to the identification of fentanyl in drug abuse cases was evaluated by developing a simpler and more rapid mass spectrometric method for identification of fentanyl in urine. Urine from six fentanyl-positive cases under review by the Office of the Chief Medical Examiner of West Virginia was included in the studies. Each of the six cases described in the presentation was investigated as an apparent drug overdose. A complete autopsy was performed on each of the decedents including comprehensive toxicology testing. Alcohol analysis was by direct injection gas chromatography with *t*-butanol as an internal standard. Drugs of abuse were screened by enzyme-multiplied immunoassay technique. Fentanyl was identified in each case by either enzyme-linked immunosorbent assay or by GC/MS. Blood fentanyl concentrations were determined on an Agilent 1100 Series LC/MSD. Chromatography was performed on a Zorbax 5SB-C_{18}, 4 x 150mm column using an isocratic solvent system (20% ammonium formate, 80% acetonitrile). The APCI interface parameters were drying gas 10 L/min, drying gas temperature 350°C, nebulizer pressure 25 psi, vaporizer temperature 300°C, and capillary voltage 4000 V. The ions monitored under SIM mode were m/z 337, 338 for fentanyl and 251, 252 for methaqualone (internal standard). A negative control consisted of pooled urine from normal healthy volunteers. To quantify fentanyl concentrations, $^{2}H_{5}$-fentanyl was used as an internal standard. Urine (1mL) samples from overdose cases were spiked with 10 µL of deuterium labeled internal standard (10 µg/mL), then filtered through a 0.2 µm PTFE membrane. A 50 µL aliquot was diluted to 200 µL total volume with 0.1% formic acid in acetonitrile. Samples were centrifuged for 5 min at 13,000 rpm. The solution was injected into the electrospray ionization (ESI) source of an ion trap mass spectrometer operating in the positive ion mode. A standard curve from control urine was constructed from spiked fentanyl HCl concentrations. Blank methanol/water mixture (50:50 v/v) was injected between two samples for cleaning purposes. Multistage mass spectra recorded in MS, MS/MS and MS/MS/MS (MS^3) modes were used to quantify and confirm the presence of fentanyl in the samples. Although present, ion suppression was not a problem at the concentrations measured above 100 ng/mL of urine.

Because the method involves direct injection into a mass spectrometer and does not require a chromatography step, considerable savings of time (3 to 4 min per sample) and costs are possible compared to the application of literature mass spectrometric methods for the identification of fentanyl. Multistage mass spectrometry methods were also developed from blood and liver for methamphetamine and MDMA.

Multistage Mass Spectrometry, Fentanyl, Forensic

K6 Determination of 2-Chloracetophenone in Air by SPME-GC/MS

*Roberto Gagliano-Candela, PhD**, and Giuseppe Strisciullo, University of Bari, Dipartimento Medicine Interna Medicina Pubblica, Policlinico, Piazza G. Cesare n.11, Bari, 70124, Italy; Stefano Dugheri, Marco Pacenti, Giulio Arcangeli, PhD, and Vincenzo Cupelli, PhD, University of Florence, Occupational Medicine Division, Department of Public Health, Largo Palagi, 1, Firenze, 50100, Italy*

After attending this presentation, attendees will understand the analysis of 2-Chloracetophenone in air by SPME extraction and GC/MS analysis.

This presentation will impact the forensic community and/or humanity by demonstrating a robust, sensitive and simple analytical method for the determination and measurement of 2-Chloracetophenone (CN) in air. Sampling by SPME requires no pumps, and no polluting organic solvents, thus reducing the sampling cost.

Laboratory and field evaluations were performed to validate the solid-phase microextraction (SPME) technique for the determination of CN in air. This is a new, rapid air sampling/sample preparation methodology suitable for use in the working environment and in forensic applications. The Threshold Limit Value (TLV)-Time Weighted Average (TWA) for CN of 0.32 mg/m^3 is recommended by the American Conference of Governmental Industrial Hygienists (ACGIH).

CN is widely used as tear gas by law enforcement agents and also by civilians for the purpose of personal protection. Recently, there has been an increase in crimes involving robbery and rape using tear gas sprays (Kataoka M. et al., J Forensic Sci 2002; 47(1): 44-51). Exposure to this lachrymator produces an intense sensory irritation of the eyes, contact dermatitis, and respiratory distress.

SPME, introduced by Pawliszyn et al. in recent years, is a solvent-free technique that combines sampling and sample preparation in a single step. The SPME sampler is a 1 cm long fused-silica fiber core coated with a polymeric phase. The coated fiber can be moved into and out of a stainless steel needle (area of needle opening, 0.00086 cm^2). By retracting the coated fiber into its needle (Z, from 1 to 35 mm) during sampling, SPME can be used as a TWA diffusive sampler.

In the present work, a method involving gas chromatography/mass spectrometry (GC/MS) and SPME was developed for quantitative analysis of CN in air. The TWA concentration of CN was analysed in a military storage facility, containing tear gas canisters, for a period from 240 to 480 min to evaluate the risk.

For laboratory validation and field TWA sampling of CN with the SPME technique, a 65 µm fiber in PDMS/DVB was used. The sampling was performed adopting a Z value of 3 mm and exposing to the air for periods of time from 60 a 480 min. After the sampling, the fiber was analysed with GC/MS.

Vapors of CN (0.032-3.2 mg/m^3) were generated by a syringe pump in a dynamic system with monitored temperature (20 and 35°C), relative humidity (10 and 80%) and air velocities (0.2 and 83 cm/s). Every thirty minutes, 200 µl of CN generated vapors were injected into the GC/MS system to monitor the dynamic system CN concentrations.

The theoretical sample rate (SR, ml/min) of CN was estimated by the Fuller-Schettler-Giddings diffusion coefficient. The experimental SR was obtained by comparing GC/MS standard solutions of CN with the amount of CN adsorbed into the fiber allowed in the sampling chamber at known concentrations.

Statistical analysis of laboratory validations demonstrated that temperature, relative humidity and air velocity did not affect the absorption efficiencies ($p<0.05$). The theoretical and experimental SR values (0.01086 and 0.00891 ml/min, respectively, at 25°C for Z=3 mm), were in good agreement. The method's precision (n=5) was established to be 10% relative standard deviation for 0.032 mg/m^3 and 8% RSD for 3.2 mg/m^3 (for 240 min sampling and Z=3 mm). The total on-column limit of quantification (LOQ) was 5 pg (0.460mg/m^3/min), and the linearity of the method ranged from 5 to 5000 pg (105 m/z).

The results obtained from the field study for the determination of the TWA concentration of airborne CN showed values ranging from 0.049 to 0.206 mg/m^3.

2-Chloracetophenone , SPME GC/MS Detection, Chemical Weapons

K7 Mass Spectrometric Data Characteristics of 7-Aminoflunitraze-pam and 7-Aminoclonazepam With Multiple Derivatization Groups

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After attending this presentation, attendees will have an enhanced appreciation of the significance and important factors associated with the selection of derivatization reagent, internal standard, and ion pairs for GC-MS analysis of drugs in biological specimen.

This presentation will impact the forensic community and/or humanity by advancing the practice in the quantification of drugs/metabolites in biological specimens.

Detecting low-levels of flunitrazepam metabolites in blood and bloodstains was reportedly facilitated by sequential derivatization with pentafluoropropionyl (PFP) and *t*-butyldimethylsilyl (TBDMS) groups [1]. Based on these findings, this study was carried out to compare the effectiveness of several groups when used in sequential derivatization of 7-aminoflunitrazepam and 7-aminoclonazepam, two benzodiazepines with more than one active site. Commercially available deuterated analogs of these two compounds, d_3-7-aminoflunitrazepam, d_7-7-aminoflunitrazepam, d_4-7-aminoclonazepam, were also included in this study to determine their effectiveness as internal standards for quantification.

Trifluoroacetyl (TFA), PFP, and heptafluorobutyryl (HFB) were adapted as the first, while trimethylsilyl (TMS) and TBDMS were used as the second derivatization groups. Products resulting from the first step and the two-step derivatization processes were analyzed by GC-MS. Full-scan mass spectrometric data were used to select ions with the potential for designating the analytes and their respective deuterated analogs in quantitative analysis protocols. Selected ion monitoring data of these ions were then collected and assessed to determine whether the quality of these ions were significantly different when one or two different derivatization groups were adapted in these sample preparation processes (Table 1). A total of 54 full-scan mass spectra and 3 ion intensity cross-contribution tables, representing various forms of derivatization and isotopic analogs of these two compounds, are systematically presented for reference. Evaluations of these data concluded: (a) for 7-aminoflunitrazepam, combination of PFP/TMS derivatization with d_7-7-aminoflunitrazepam serving as the internal standard generated the most favorable ion pairs for quantification and as supporting parameters for qualitative analysis purposes; (b) data resulting from the 7-aminoclonazepam study were not as clear; however, the combination of TFA/TMS appeared to be the best choice.

Reference:

1. A.A. Elian. Detection of low levels of flunitrazepam and its metabolites in blood and bloodstain. Forensic Sci. Int. 101 (1999) 107–111.

Table 1. Double derivatization groups, most favorable ions (m/z) for designating the analytes and their deuterated internal standards, and percent cross-contribution by the internal standard to the intensity of ions designated for the analyte and vise versa.

Derivatization Group[a]	Ions (and % cross-contritbution) designating analyte and internal standard d_0- and d_7-7-aminoflunitrazepam	 d_0- and d_4-7-aminoclonazepam
Ethyl/ethyl	—[b]	312 (1.70), 341 (4.78), 342 (1.41)
	—	316 (6.32), 345 (6.87), 346 (2.29)
Propyl/propyl	—	340 (0.88), 369 (0.31), 370 (0.75)
	—	344 (2.71), 373 (1.00), 374 (0.34)
Butyl/butyl	—	354 (0.38), 397 (0.23), 398 (0.64)
	—	358 (0.90), 401 (1.18), 402 (0.35)
TMS/TMS	—	394 (0.36), 414 (0.23), 429 (0.33)
	—	398 (2.53), 418 (6.00), 433 (6.47)
t-Butyl-TMS/*t*-butyl-TMS	—	456 (0.38), 457 (0.61), 458 (1.01)
	—	460 (6.59), 461 (3.84), 462 (0.77)
TFA/TMS	423 (0.35), 450 (0.43), 451 (0.44)	—
	430 (0.08), 456 (0.50), 458 (0.00)	—
TFA/*t*-butyl-TMS	436 (0.16), 437 (0.20), 493 (0.15)	—
	443 (0.01), 444 (0.28), 500 (0.00)	—
TFA/2*t*-butyl-TMS	—	552 (1.52), 553 (1.61), 554 (2.00)
	—	556 (4.11), 557 (2.33), 558 (0.42)
PFP/TMS	473 (0.17), 500 (0.24), 501 (0.17)	—
	480 (0.64), 506 (0.05), 508 (0.00)	—
PFP/*t*-butyl-TMS	337 (6.58), 486 (5.45), 543 (5.72)	—
	340 (2.49), 493 (0.02), 550 (0.00)	—
PFP/2*t*-butyl-TMS	—	602 (0.13), 603 (0.24), 604 (0.51)
	—	606 (6.71), 607 (4.75), 608 (0.85)
HFB/TMS	523 (0.37), 550 (0.27), 551 (0.27)	—
	530 (6.10), 556 (0.09), 558 (0.00)	—
HFB/*t*-butyl-TMS	296 (4.81), 536 (3.00), 537 (3.10)	—
	299 (1.92), 543 (0.22), 544 (0.43)	—
HFB/2*t*-butyl-TMS	—	652 (0.27), 653 (0.32), 654 (0.67)
	—	656 (4.97), 657 (2.57), 658 (0.63)

[a] TMS: trimethylsilyl; *t*-butyl-TMS: *t*-butyldimethylsilyl; TFA: trifluoroacetyl; PFP: pentafluoropropionyl: FFB: heptafluorobutyryl.

[b] Attempts to attach the second derivatization group were unsuccessful.

Flunitrazepam, Clonazepam, GC-MS

K8 Simultaneous Determination of HFBA-Derivatized Amphetamines and Ketamines in the Urine by GC-MS

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After attending this presentation, attendees will learn about a new method for HFBA derivatives for amphetamines and ketamine and its metabolites using GC-MS.

This presentation will impact the forensic community and/or humanity by improving analytical cost and time.

This study developed a rapid, sensitive, and accurate method for the simultaneous determination of 8 commonly abused drugs/metabolites containing amine functional groups, i.e., amphetamine, methamphetamine, MDA, MDMA, MDEA, ketamine, norketamine and dehydronorketamine. The protocol included solid phase extraction, HFBA derivatization and GC-MS analysis, using d_5-amphetamine, d_8-methamphetamine, d_5-MDA, d_5-MDMA, d_6-MDEA, d_4-ketamine and d_4-norketamine as the internal standards. Identification of these compounds was based on retention time information and the relative abundance of the following ions established for each analyte as derivatized by HFBA: amphetamine: 240, 118, 91; methamphetamine: 254, 210, 118; MDA: 135, 162, 239; MDMA: 254, 162, 210; MDEA: 268,162, 240; ketamine: 210, 236, 370; norketamine; 384, 356, 377; dehydronorketamine: 314, 382, 169. The following analytical parameters have also been established: linear range: 100–2000 ng/ml; limits of detection and quantitations (all in ng/ml): 60 and 75 for amphetamine; 60 and 75 for methamphetamine; 75 and 100 for MDA; 75 and 100 for MDMA; 75 and 100 for MDEA; 30 and 50 for ketamine; 50 and 75 for norketamine and 50 and 125 for dehydronorketamine. The overall method recoveries of HFBA-derivatized amphetamine analogs were 92–99%, with less than 5% CV of intra-day and inter-day data. In conclusion, this method provides a uniform procedure for confirmation tests of the amphetamines and ketamine drug categories under workplace drug testing settings. Under clinical testing environment, it can be effectively used for the preliminary and confirmatory testing of these 8 drugs/metabolites, without the need for screening by three separate immunoassays, specific for amphetamine/methamphetamine, MDA/MDMA/MDEA, and ketamines, respectively.

Ketamines, Amphetamines, GC-MS

K9 Quantitation of Quetiapine in Human Blood by Solid Phase Extraction and High-Performance Liquid Chromatography

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After attending this presentation, attendees will learn a simple but effective means of identifying and quantitating quetiapine in blood that can be implemented into their own laboratories.

This presentation will impact the forensic community and/or humanity by providing a simple, sensitive, and selective means of identifying and quantitating quetiapine at a range of therapeutic, toxic and fatal blood concentrations.

Quetiapine is an atypical antipsychotic drug indicated as monotherapy for the management of manic episodes associated with bipolar disorder and for the treatment of schizophrenia. Therapeutic concentrations of quetiapine have been reported to range from approximately 0.2 to 1 mg/L. Fatalities attributed to quetiapine overdose have been reported to occur at blood concentrations of 7 mg/L and greater. The incidence of detection of quetiapine, or its indication in case history in death investigations in Ontario, has increased progressively on a year-to-year basis between 1998 and 2004. Therefore, a sensitive and selective high-performance liquid chromatography (HPLC) assay employing solid phase extraction (SPE) has been developed and validated to analyze for quetiapine over a forensically-relevant range of blood concentrations. Selective detection of the analyte is achieved by utilizing an ultraviolet photodiode array detector (UV-DAD) to identify the distinctive UV spectra of quetiapine at a monitoring wavelength of 215 nm at the appropriate retention time. Carbinoxamine maleate, an antihistamine marketed in the United States but is not available in Canada, is used as an internal standard at a concentration of 1.0 mg/L. The limit of detection for quetiapine in this assay is 0.03 mg/L with a lower limit of quantitation of 0.125 mg/L. This method provides a linear response to quetiapine concentrations ranging from 0.125 to 4 mg/L, above which the sample can be diluted and quantitated using an external calibration curve. The extraction recoveries of quetiapine and carbinoxamine were 70±10% and 84±6% (mean± S.D.), respectively. Intra-assay linear regression analysis of the calibration curves in blood (n=5) had r^2 values ranging from 0.987 to 1.00. Inter-assay linear regression analysis of the calibration curves in blood (n=6) had r^2 values ranging from 0.990 to 0.999. The intra-assay precision in blood calibration standards (n=5) at each calibration level ranged from 4 to 8% relative standard deviation (RSD) over the concentration range 0.125 to 1.0 mg/L. The inter-assay precision in blood calibration standards over six days ranged from 6 to 9% RSD at each calibration level over the concentration range 0.125 to 1.0 mg/L. As a measure of accuracy, the percent difference from target concentrations ranged from 0 to 11% (mean 6%) based on the analysis of two internally-prepared, single-blind samples (0.25 and 0.50 mg/L) and one zero-blind sample (1.0 mg/L). This assay provides a simple, sensitive and selective means of identifying and quantitating quetiapine over a range of therapeutic, toxic, and fatal blood concentrations.

Quetiapine, HPLC, Solid Phase Extraction

K10 Detection of Benzoylecgonine in Urine Using the V-Flex System

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The goal of this presentation is to provide results of a validation study regarding the analysis of benzoylecgonine in urine using an automated solid-phase extraction system (V-Flex).

This presentation will impact the forensic community and/or humanity by evaluating an automated solid-phase extraction system, which provides rapid throughput for an increased sample load with minimal manual labor.

Solid-phase extraction (SPE) is a widely accepted isolation technique utilized for the analysis of drugs and drug metabolites in urine. BioIntegrated Solutions (Palatine, IL) is currently developing the V-Flex, an automated SPE system. This study evaluates the use of this system for the analysis of benzoylecgonine (cocaine metabolite) in urine.

Benzoylecgonine and its deuterated analog (internal standard), d_3-benzoylecgonine (Cerilliant Corporation, Round Rock, TX), were extracted from urine utilizing the V-Flex automated solid-phase extraction system. Prior to extraction, urine specimens were diluted in 0.1 M phosphate buffer (pH 6.0), alkalinized with 1 N NaOH, centrifuged, transferred to a clean glass culture tube, and submitted to the V-Flex system. With minimal manual intervention, the automated system conditioned the SPE copolymeric bonded phase cartridges (United Chemical Technologies, Inc., Bristol, PA), transferred specimens, performed washes, and eluted the desired compounds with ethyl acetate/methanol/ammonium hydroxide (68/28/4) elution solvent. The extracts were dried under a stream of

nitrogen at 50°C, derivatized with N-methyl-N-(tert-butyldimethylsilyl)trifluoroacetamide (MTBSFTA), and analyzed with an Agilent 5890 Series II Gas Chromatograph (GC) system equipped with a 5972 Series Mass Selective Detector (MSD) (Little Falls, DE). The GC was fitted with a Restek Rtx-5 capillary column (30 m x 0.25 mm x 0.10 μm) (Bellefonte, PA) with ultra-high-purity helium as the carrier gas at a constant flow rate of 1.0 mL/min. Automated injections were made in splitless mode. The mass spectra were obtained in selected ion monitoring mode by monitoring *m/z* 282.2, 346.2, and 403.2 for benzoylecgonine and *m/z* 349.2 and 406.2 for deuterated benzoylecgonine.

The automated SPE protocol was compared to a manual SPE method employed in the laboratory. Minor differences in the manual method include solvent volumes, an additional wash step with acetonitrile, and the elution solvent utilized was methylene chloride/isopropanol/ammonium hydroxide (78/20/2). Finally, the manual method employed a five-point calibration curve.

Validation studies utilizing one-point calibration at 150 ng/mL and control concentrations of 120, 180, and 500 ng/mL demonstrated intra-assay and inter-assay % CV values that were less than 3%, and intra-assay and inter-assay % accuracy values within 11%. The range of linearity was 75-750 ng/mL. Analysis of authentic urine specimens by the automated SPE and manual SPE operating procedures produced excellent correlation. Initial studies have demonstrated a correlation coefficient of 0.95.

In conclusion, automated SPE using the V-Flex system is an efficient method for the analysis of benzoylecgonine in urine.

Benzoylecgonine, Automated Solid-Phase Extraction, GC-MS Analysis

K11 Methadone to Metabolite Ratio in Cases of Fatal Overdose

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The goal of this presentation is to provide the attendees with information that is pertinent to the interpretation of methadone and methadone metabolite results in deaths that are due to methadone intoxication.

This presentation will impact the forensic community and/or humanity by demonstrating assisting forensic toxicologists and pathologists in evaluating methadone and methadone metabolite blood concentrations in cases of fatal methadone intoxication.

Identifying that a death has occurred due to accidental drug overdose requires consideration of a host of factors. The phenomenon of pharmacodynamic (cellular) tolerance is of particular significance in evaluating opioid blood concentrations in circumstances that suggest fatal overdose. Accordingly, opioid concentrations in those who have died from causes other than overdose are, for the most part, indistinguishable from those found in fatal overdose. With the recent increase in the prevalence of methadone in many areas, correctly identifying the extent to which methadone is causally related, whether as the sole agent in a fatal overdose, or its significance as a contributing factor in a multiple-drug overdose, is of increasing importance.

For many drugs, the concentration of parent drug relative to that of one or more metabolites provides an indication of the extent to which a drug was used during a period of time preceding death. With opioid drugs, awareness of their use prior to death is useful in assessing the degree to which the user may have developed cellular tolerance. A study published in 1988 by Hartman et al. (1) addressed the use of a propoxyphene metabolite as a means of evaluating chronic use, and hence, tolerance. Similarly, the purpose of this study is to assess the relationship between methadone and its principle metabolite, EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine), in cases of fatal overdose in which no alcohol or other drugs were detected. As an adjunct to case information about previous drug use, the role of the metabolite, EDDP, in relation to parent drug, methadone, is suggested. EDDP is formed by spontaneous cyclization following cytochrome P450-mediated *N*-demethylation of methadone.

Each case considered for inclusion in the study received a full autopsy performed at the West Virginia Office of the Chief Medical Examiner. Toxicological analysis was performed to determine the presence of volatiles in blood by direct injection GC-FID, drugs of abuse screening of blood and/or urine by enzyme-multiplied immunoassay technique (EMIT), and GC-MS screening of acidic/neutral and basic drugs in blood and/or basic drugs in urine. Positive drug screening results were confirmed and quantitated by GC-MS or LC-MS. Methadone and EDDP concentrations were determined in subclavian blood in each case by GC-MS using SKF-525A as internal standard.

Data included in the study was limited to consecutive cases found to be "methadone only" drug overdoses which occurred in West Virginia between January of 2003 and July of 2005. During this time period, 21 deaths due to methadone intoxication were identified of which fourteen of the decedents were male and seven were female. Methadone concentration in subclavian blood averaged 665 ng/mL $\pm$ 470 ng/mL and ranged from 98 ng/mL to 1846 ng/mL. Average EDDP concentration was 48.2 ng/mL $\pm$ 39.3 ng/mL and ranged from 5 ng/mL to 150 ng/mL. The average ratio of blood methadone concentration to EDDP concentration was 16.1 $\pm$ 5.8 with a range of 7.9 - 29.4. EDDP concentration was found to be correlated with that of methadone, $r^2 = 0.82$ ($p < 0.01$). A previous study has shown that methadone can be converted to EDDP as an analytical artifact due to an elevated gas chromatograph injector port temperature (2). The method of analysis also resulted in methadone conversion to EDDP, but was found to be less than 1.0% of the methadone concentration.

Consistent with previous reports, these data demonstrate that methadone blood concentrations in fatal overdose vary enormously. The ratio of methadone to EDDP may, however, provide additional information in establishing overdose in cases where overdose is supported by case information and no drugs other than methadone and its metabolite(s) are found. Methadone has a longer half-life than most other opioid drugs. For EDDP concentration to be useful as an indicator of chronic methadone use, it would need to be shown that it has a long half-life and that its concentration becomes elevated with chronic methadone use. At present, however, EDDP's half-life has not been clearly demonstrated. To more thoroughly affirm EDDP concentration or the methadone to EDDP ratio as potential indicators of tolerance, further study is needed of parent drug and EDDP concentrations in deaths in which the decedent was positive for methadone, but methadone was not a contributory factor in the death.

References:
1. B. Hartman, D.S. Miyada, H. Pirkle, P. Sedgwick, R.H. Cravey, F.S. Tennant and R.L. Wolen. Serum propoxyphene concentrations in a cohort of opiate addicts on long-term propoxyphene maintenance therapy. Evidence for drug tolerance in humans. *J. Anal. Toxicol.* 12:25-29, 1988.
2. F.R. Galloway and N.F. Bellet. Methadone conversion to EDDP during GC-MS analysis of urine samples. *J. Anal. Toxicol.* 23:615-619, 1999.

Methadone, Opioid, Overdose

K12 Childhood Prilocaine Fatality

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The goal of this presentation is to inform the attendees of the circumstances and toxicology involving the death of a child that resulted from inadvertent excessive administration of the local anesthetic prilocaine during a dental procedure.

This presentation will impact the forensic community and/or humanity by emphasizing the need for extreme caution when administering a local anesthetic to a patient with a low body mass, such as a child.

Severe toxicity from local anesthetics used in dental procedures is often due to accidental intravascular injection. To lessen the likelihood of such an occurrence, aspiration is performed before the anesthetic solution is injected. In the event that blood is aspirated, the needle is repositioned until no blood is observed upon aspiration. Nevertheless, rigorous adherence to such a precautionary measure, although fairly preemptive, does not definitively abolish inadvertent intravascular injection.

Among the local anesthetics commonly used in dental procedures is prilocaine or Citanest® (AstraZeneca Pharmaceuticals, Wilmington, DE). As with other local anesthetics, the pharmacological activity of prilocaine is mediated by blockage of voltage gated sodium channels. Administration of local anesthetics involves injection into the region of the nerve fibers to be blocked. The onset of anesthesia occurs an average of two minutes following prilocaine injection and lasts for approximately two hours. Prilocaine has a volume of distribution of 0.7-4.4 L/kg with 30% of the plasma concentration bound to proteins [(1)].

In preparation for a dental extraction procedure, a healthy 2-year-old male was administered nitrous oxide for sedation. This was followed by injection of four 1.8 mL ampules of the local anesthetic, prilocaine, with another ampule applied topically. Shortly thereafter, the child became quiet, exhibited seizure-like activity and became cyanotic. The child's condition improved following administration of 100% oxygen. However, upon arrival at the hospital, he went into cardiopulmonary arrest and was pronounced dead approximately 85 minutes after conclusion of prilocaine delivery. Routine toxicology screening analysis of blood and urine revealed the presence of only prilocaine and lidocaine. Anaphylactic reactions to amide-type local anesthetics are rare and measurement of serum tryptase, an indicator of anaphylaxis, was negative. Prilocaine concentration was measured by GC-MS with SKF-525A as internal standard. Blood obtained from the subclavian vein and the heart contained prilocaine at 14.6 and 13.0 mg/L, respectively. Concentrations of prilocaine in additional samples obtained at autopsy are indicated in Table 1. The cause of death was determined to be prilocaine toxicity resulting from excessive administration by injection. Prilocaine was measured in several other samples obtained at autopsy with the results reported herein. Kaliciak and Chan reported the death of an elderly patient undergoing a dental procedure with the blood prilocaine concentration of 13.4 mg/L, very similar to that found in the present fatality [(2)].

Table 1

Heart blood	13.0 mg/L
Peripheral blood	14.6 mg/L
Liver	14.0 mg/kg
Lung	26.1 mg/kg
Bile	31.1 mg/L
Vitreous fluid	14.7 mg/L
Urine	12.4 mg/L
Gastric contents	76.4 mg/L

As with all drug administration in the pediatric age group, the maximum dosage of prilocaine that may be safely delivered is governed by the weight of the child. Based on the manufacturer's recommendation of a maximum dose of 8 mg per kilogram, the total administered dose of prilocaine to this child, who weighed slightly less than 15 kilograms, would be limited to 120 mg of prilocaine (15 kg x 8 mg/kg). Delivery of such a dose corresponds to a total of 3 mL of prilocaine solution; available only as a 4% solution. As such, a maximum of one and two-thirds dental cartridges of prilocaine should be administered simultaneously to a child of this weight. This indicates that the significantly elevated concentration of prilocaine in this child is the result of excessive administration of this local anesthetic rather than a complication of direct intravascular bolus administration; a conclusion that is further supported by the dentist's account of the delivery of the prilocaine injections.

References:

1. R.C. Baselt. *Disposition of Toxic Drugs and Chemicals in Man,* 7th ed., Biomedical Publications, Foster City, CA, 2004, pp 929-930.
2. H.A. Kaliciak and S. C. Chan. Distribution of prilocaine in body fluids and tissues in lethal overdose. *J. Anal. Toxicol*. 10: 75-6 (1986).

Prilocaine, Fatality, Anesthetic

K13 Case Report: Death Due to Snorting of Crushed Sustained-Release Morphine Tablets

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After attending this presentation, attendees will learn about potentially fatal low blood morphine concentrations that are not heroin-related can exist, especially if the route of administration and drug formulation administered are unusual.

This presentation will impact the forensic community and/or humanity by providing a case report of a death due to snorting of a sustained-release morphine formulation, especially one where heroin is not a potential confound. This case report will therefore address this specific absence in the literature.

MS-Contin® is a sustained-release morphine formulation that is administered orally to treat moderate to severe pain. MS-Contin® is available in tablets containing 15, 30, 60, 100 and 200 mg of morphine sulfate. Within four hours of the administration of 30 or 60 mg tablets of MS-Contin®, the reported peak plasma morphine concentrations are 10 and 30 ng/mL, respectively. Therapeutic plasma morphine concentrations persist for about 12 hours thereafter.

This report documents a morphine-related death in a male prisoner known to be an intravenous drug user who reportedly snorted three crushed 100 mg tablets of MS-Contin® in his jail cell. The prisoner died 8 hours later. Prior to death this individual exhibited symptoms of profound sedation and laboured breathing that progressed to apnea. At autopsy the pathologist observed pulmonary edema. In addition, two condoms were found in his rectum, one containing three 100 mg tablets of MS-Contin®, the other containing plant material suspected of being marijuana. Absorption of morphine from the condom was ruled out based on a visual assessment of condom integrity and the condition of the tablets. Toxicological examinations of post-mortem blood and urine samples were conducted to determine whether death was related to the presence of illicit substances or pharmaceutical preparations often encountered in death investigations. Analysis for heroin was not performed as there was no investigative information to suggest its use. The analytical procedures consisted of immunoassays and gas chromatographic methods, utilizing flame ionization, nitrogen-phosphorus, and mass spectrometric detection. A concentration of 103 ng/mL of free morphine was detected in the femoral blood, and cannabinoid metabolites were indicated by an immunoassay in heart blood. No alcohol, or other substances of toxicological significance were detected.

The reported symptoms, autopsy findings, and the results of the toxicology examination point to a fatal morphine overdose. In the experience of this laboratory, this is the first known death associated with the snorting of a crushed sustained-release morphine tablet.

Morphine, Snorting, Fatal

K14 Serum and Blood Concentrations of the Oxcarbazepine (Trileptal®) Metabolite, 10-Hydroxy-Carbazepine

Lee M. Blum, PhD, Erica L. Horak, BS, and Robert W. Dalrymple, BA, National Medical Services, Inc., 3701 Welsh Road, Willow Grove, PA 19090*

After attending this presentation, attendees will learn about the observed serum/plasma and blood concentrations of 10-hydroxycarbazepine, the active metabolite of oxcarbazepine (Trileptal®), in a patient population.

This presentation will impact the forensic community and/or humanity by providing a review of such a population is important as either an elevated or a sub-therapeutic circulating level of anticonvulsant drugs such as oxcarbazepine can be a significant finding in a forensic investigation.

Serum and blood concentrations from over 53,000 specimens were reviewed for the 10-hydroxy-carbazepine metabolite of oxcarbazepine (Trileptal®). Oxcarbazepine is an anticonvulsant drug used for the treatment of partial seizures alone or as adjunct therapy in adults and as add-on therapy in children ages 4 to 16 with epilepsy. Although it is chemically similar to carbamazepine, its metabolism is different. Following administration, oxcarbazepine is rapidly reduced to 10-hydroxy-carbazepine which is primarily responsible for the anticonvulsant activity of the drug. It is available as 150 mg, 300 mg and 600 mg filmed capsules for oral administration. In adults, 1200 mg/day and 2400 mg/day are typically administered for adjunct therapy and monotherapy, respectively. In children, depending on their weight up to 1800 mg/day can be given. It's recommended that all doses be given in a twice a day regimen. Peak concentrations following a single dose are within 1-3 hours for oxcarbazepine and 4-12 hours for the metabolite. Steady state plasma concentrations of 10-hydroxy-carbazepine are usually achieved in 2 to 3 days. The half-life of the parent is approximately 2 hours while that of the metabolite is about 9 hours. The suggested target concentrations for therapeutic monitoring of 10-hydroxy-carbazepine have been reported to be approximately 13 – 35 mcg/mL. Common adverse effects related to oxcarbazepine therapy included dizziness, somnolence, diplopia, fatigue, nausea, vomiting and ataxia among others. A review of patient samples was performed to determine the observed ranges of serum/plasma (n=53,485) or blood (n=174) concentrations in a patient population. Because samples were received from other testing facilities, no histories or dosing regimens were provided. The analyses were performed by HPLC with a reporting limit of 0.5 mcg/mL. In the serum/plasma population, 2154 samples had no 10-hydroxy-carbazepine detected. Of those patients with oxcarbazepine metabolite found, the concentration ranged from 0.5 to 110 mcg/mL with a mean = 16.9 ± 9.6 mcg/mL and a median = 16.0 mcg/mL. In those samples where blood was tested, 32 were none detected and the remaining patient samples had a mean = 18.9 ± 21.9 mcg/mL (range 0.5 – 140 mcg/mL) and a median = 14.5 mcg/mL. Approximately 60% of the serum/plasma samples, where 10-hydroxy-carbazepine was reported, were within the targeted therapeutic range of 13 – 35 mcg/mL, while about 45% of the blood samples were within this range. The percentage of samples greater than 35 mcg/mL was 4.2% for the serum/plasma samples and 9.9% for the blood samples. The serum/plasma concentrations with the highest frequencies of samples ranged between 9 and 18 mcg/mL. Although many factors may have influenced the concentrations observed, a review of such a population is important as either an elevated or a sub-therapeutic circulating level of anticonvulsant drugs such as oxcarbazepine can be a significant finding in a forensic investigation.

Oxcarbazepine, 10-Hydroxycarbazepine, Serum/Blood Concentrations

K15 Inhalant Abuse Involving Difluoroethane

Douglas E. Rohde, MS, Lake County Crime Laboratory, 235 Fairgrounds Road, Painesville, OH 44077; and Dwight Flammia, PhD, Department of Forensic Science, 700 North 5th Street, Richmond, VA 23219*

After attending this presentation, attendees will learn of two cases of intentional inhalation involving a readily available compound and the methods used to identify this compound.

This presentation will impact the forensic community and/or humanity by providing examples of non-fatal and fatal inhalation of difluoroethane and increasing the awareness of inhalant abuse.

1,1-Difluoroethane (DFE) is a colorless gas with a slight ethereal odor used in aerosol preparations and coolants. DFE can produce headache, weakness, dizziness, nausea, confusion, labored breathing, lung irritation, loss of consciousness, and cardiac arrhythmia. Overexposure may result in fatality due to displacement of oxygen.

The first case involves the intentional abuse of DFE by a motorist. A 32-year-old white male was observed slumped over behind the wheel of a stopped vehicle. Several aerosol cans labeled Endust for Electronics® were observed lying on the floor of the vehicle. After the subject was roused, he admitted he had been "huffing", stating he had consumed one aerosol can and was starting on another. Subject's face was red with watering eyes. A rapid head movement from left to right was also observed. Subject was placed under arrest for DUID and transported to a medical center where blood was collected.

Qualitative headspace analysis of whole blood samples as well as one of the suspect aerosol cans by gas chromatography-mass spectrometry (GCMS) indicated the presence of DFE. A standard was prepared by introducing propellant from an Endust for Electronics® can into a 20 ml headspace vial rapidly sealed. Confirmation of DFE was accomplished by the comparison of blood sample spectra to DFE standard spectra. No other toxicology analyses were performed.

The second case involves the intentional inhalation of DFE by a high school student. The decedent was a 14-year-old white male found by his mother lying motionless in his bed with his legs crossed and his head leaning to one side. An aerosol can labeled Dust-Off® was found in his hands with the delivery tube still in his mouth. Further investigation revealed the decedent had previously inhaled Dust-Off® with friends who referred to the practice as "dusting". A subsequent search of a neighboring school yielded the discovery of another aerosol dust remover labeled Clean Safe® containing 1,1,1,2-Tetrafluoroethane (TFE) in the possession of a student.

The father of the decedent was a police officer, the mother a nurse. A German shepherd police dog trained in the detection of drugs lived with the family. The decedent's father revealed that his son had experienced one episode of vomiting the week before and complained once of a numb tongue. Specimens obtained at autopsy included cardiac blood and femoral blood collected in sealed polypropylene vials as well as lung tissue from each lung and a tracheal air sample collected in 20 ml headspace vials. Specimens were stored at 4º C until analysis.

Specimens were submitted for toxicology testing, including a volatile screen by headspace gas chromatography with flame ionization detection and a drugs of abuse screen utilizing an enzyme-linked immunosorbent assay. The volatile screen on femoral blood indicated the presence of DFE while the drugs of abuse screen were negative. Confirmation of DFE was accomplished by qualitative headspace analysis by GCMS. A standard was prepared by introducing propellant from a Dust-Off® can into a 20 ml headspace vial rapidly sealed. DFE was identified in the cardiac blood and both lung samples. The tracheal air sample was negative. The cause of death was determined to be chemical asphyxia and the manner of death accidental.

According to the American Academy of Pediatrics, the peak age of inhalant abusers is 14 to 15 years, with onset occurring in those as young as 6 to 8 years. Use of inhalants typically declines by 17 to 19 years.

Difluoroethane, Headspace, Inhalant Abuse

K16 Fentanyl Concentrations in 23 Postmortem Cases From Hennepin County Medical Examiner's Office

Jonathan G. Thompson, MD, Andrew M. Baker, MD, Julie S. Kloss, MBA, Quinn Strobl, MD, and Fred S. Apple, PhD, Hennepin County Medical Center, Clinical Laboratories, P4, 701 Park Avenue South, Minneapolis, MN 55415*

After attending this presentation, the attendee will have better interpretability of postmortem blood fentanyl concentrations and its role in one's death.

This presentation will impact the forensic community and/or humanity by improving the understanding of postmortem blood fentanyl concentrations and showing the importance of the deceased's past medical history in signing out the cause and manner of death.

The purpose of this study was to compare blood fentanyl concentrations in fentanyl-related deaths with fentanyl concentrations found incidentally at autopsy, as well as with fentanyl concentrations found in hospitalized patients receiving fentanyl. A retrospective study, between the years 1995 to 2005, of postmortem cases from the Hennepin County Medical Examiner's Office was conducted in which fentanyl was detected. Gas chromatography – mass spectrometry was used to quantify all fentanyl levels. Of the 23 postmortem cases in which fentanyl was identified, 19 (82.6%) were deemed to be drug overdoses. Fentanyl, alone, was responsible for 7 of the 19 (36.8%) overdose deaths. Mean and median fentanyl concentrations were 38.7 µg/L and 25 µg/L, respectively, with a range of 5 to 120 µg/L. Six of the cases were signed out as accidental, one as undetermined. The remaining 12 of the 19 (63.1%) cases were mixed drug overdoses, predominantly including other opiates, barbiturates, benzodiazepines, and alcohol. Mean and median fentanyl concentrations were 30.8 µg/L and 13.5 µg/L, respectively, with a range of 5 to 152 µg/L. All of the mixed drug overdoses were signed out as accidental. Four cases where fentanyl was an incidental postmortem finding were all signed out as natural deaths; blood concentrations in this group were 2, 2, 2, and 15 µg/L. The deceased with the blood fentanyl concentration of 15 µg/L was being treated for chronic pain related to metastatic squamous cell carcinoma of the head and neck. This fentanyl level was greater than or equal to three of the fentanyl-only overdose deaths and seven of the mixed drug overdose cases.

For comparison, 11 inpatients receiving fentanyl were identified over one 24-hour period. Two of the patients had fentanyl concentrations of 8.5 µg/L and 9.9 µg/L; these levels were higher than one of the fentanyl-only related deaths (5 µg/L) and two of the mixed drug overdose cases (5 µg/L and 7 µg/L). Both patients had been receiving opiates, including fentanyl, for chronic pain for more than three months. The other nine inpatient concentrations were less than 4 µg/L.

This study shows higher mean and median blood fentanyl concentrations in cases where fentanyl alone was determined to be the cause of death when compared to cases where fentanyl was part of a mixed drug overdose. There is considerable overlap between fentanyl concentrations in fentanyl-related deaths and fentanyl concentrations in hospitalized patients being treated for chronic pain. The interpretation of fentanyl concentrations in postmortem cases must be interpreted in context of the deceased's past medical history and autopsy findings.

Fentanyl, Postmortem, Chronic Therapy

K17 HS/GC Determination of Volatile Substances in Antemortem and Postmortem Blood and Urine Samples of Volatile Substance Abusers

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After attending this presentation, the results of Headspace Gas Chromatographic analyses for volatile substances in blood and urine from three groups represented by: 23 abusers of volatile substances which applied to volatile abusers hospital AMATEM-Istanbul; 6 cases of questionable death which were autopsied at the council of forensic medicine; and 10 non-abusers in Turkey will be available.

This presentation will impact the forensic community and/or humanity by providing data on volatile substance abuse in Turkey.

Volatile Substances are known to be inexpensive, easily and legally acquired and therefore have widespread use among youngsters. The mostly widely abused volatile substance in Turkey is toluene. Toluene is extensively used as an organic industrial solvent in paint thinner, detergents and glue. Chronic exposure to low concentrations of toluene causes impairment of the central nervous system. The recommended threshold limit value-time weighted average is 50 ppm for preventing such effects. Toluene is eliminated via exhaled air and as intact compound or its metabolites, hippuric acid and *o*-cresol, in urine. Therefore, indicators of toluene exposure are, beside toluene itself in exhaled air, blood and urine, urinary hippuric acid and *o*-cresol. Additionally, volatile substance abuse (VSA), including paint thinner abuse, represents an important health threat in Turkey. Some paint thinners used in Turkey are mixtures of solvents consisting of toluene as the major component in addition to benzene, hexane and heptane.

In this study, analysis of blood and urine for volatile substances from three groups: 23 abusers of volatile substances which applied to the Volatile Abusers Hospital (AMATEM)-Istanbul; 6 cases of questionable death which were autopsied at the Council of Forensic Medicine; and 10 non-abusers were carried out and compared by using Headspace Gas Chromatography.

The analyses of volatile substances were carried out in lithium-heparinized blood and unspiked urine for the solvents used by abusers according to the method described by Park SW. et al. (J. Forensic Sci. 43:888-890 [1998]). Blood toluene content of 23 antemortem blood samples, taken 24 hour after volatile substance sniffing, were 0.96, 1.49, not detected (ND), 1.33, ND, ND, 0.99, ND, ND, 2.80, 1.35, 2.39, 1.09, 6.56, 3.88, ND, ND, 5.49, 0.66, 0.81, ND, 0.38, 2.17 with the average being 1.61µg/ml ± 1.01 (SD) (range 0.38 to 3.88). Toluene and other solvents were not measurable in urine in the majority of cases; but in four cases, urinary toluene was measured as 0.89, 069, 032 and 0.35 µg/ml. None of the samples were found to contain measurable amounts of benzene, hexane or heptane. Toluene in postmortem samples was distinguishable from that in non-fatal abusers.

Conclusion: From the analysis of blood and urine for volatile substances by Headspace Gas Chromatography, three groups represented by: 23 volatile substances abusers which applied to the Volatile Abusers Hospital (AMATEM)-Istanbul; 6 cases of questionable death which were autopsied at the morgue of The Council of Forensic Medicine; and 10 non-abusers, showed only toluene. The amount of inhalation could not be calculated or explained since the chemical compositions of the abused thinners were not consistent.

Volatile Substance Abuse, Headspace GC, Toluene

K18 Comparative Analysis of GHB and GHV I

Lucy S. Oldfield, MS, Jennifer W. Mercer, BS, Suzanne C. Bell, PhD, Jeffrey L. Petersen, PhD, Diaa M. Shakleya, PhD, and Joshua A. Gunn, BS, West Virginia University, Bennett Department of Chemistry, 217 Clark Hall, Morgantown, WV 26506*

The goal of this presentation is to summarize tests used in the detection of gamma-hydroxybutyrate (GHB) and to apply these tests to the analysis of gamma-hydroxyvalerate (GHV), an emerging drug threat. It will focus on both screening and confirmatory tests and complements the Scientific Session "Comparative Analysis of GHB and GHV II".

This presentation will impact the forensic community and/or humanity by bringing to attention the potential use of GHV as a date rape drug and outlines methods for its detection. The study offers details of simple crystal tests which are rapid and easy and can be applied to the development of a simple field test for the detection of GHB, GHV their analogs and precursors.

The illicit use of GHB and its precursors is well known and reported incidents of its exploitation as a date rape drug have increased worldwide. GHV, a 4-methyl-substituted analog of GHB, is reportedly used as an alternative to GHB and is commercially available as a dietary supplement and replacement for GHB. The behavioral effects of GHV are similar to GHB as both drugs cause sedation, catalepsy, and ataxia, however GHV requires larger doses to produce these effects. The inherent toxicity of GHV appears to be significantly higher than GHB, increasing concerns over abuse and making its detection and characterization an important issue in forensic toxicology and solid dose analysis. Like GHB, GHV is often used and abused in recreational settings and is frequently mixed with water or alcoholic beverages requiring fairly low doses commonly between 3 – 8g, correlating to between 0.8% - 2.3% w/v in a 12oz (355ml) serving.

The work to be presented here has two aspects; first, the application of presumptive tests for the screening of GHV and its precursor gamma-valerolactone (GVL) and second; the development of a head space solid phase micro extraction-gas chromatography-mass spectrometry (SPME-GC-MS) method for the confirmation of GHV and GVL. Methods used for GHB determination were applied as a basis for GHV method development.

A series of presumptive screening tests were evaluated for GHV. Both thin layer chromatography (TLC) and microcrystal tests were developed using a silver nitrate/copper nitrate mix, a reagent previously reported in the literature. Distinct crystals were observed for GHV with $Ag(NO_3)/Cu(NO_3)_2$ reagent. Infrared (IR) and Raman spectroscopy and x-ray crystallography were used for structural determination of crystals. The resulting structure was a planar, stacked crystal lattice with a silver backbone.

Confirmatory analysis was carried out using SPME-GC-MS. As the compounds of interest are small and thermally unstable under high temperature conditions a method was developed for the detection of derivatized GHV and GVL. The derivatizing agent used was N, O-bis(trimethylsilyl)triflouroacetimide (BSTFA) with trimethylchlorosilane (TMCS). SPME was carried out using a 50µm carbowax™/templated resin (CW/TPR) fiber mounted in a manual SPME holder. The fiber was adapted for GC injection by adding a spring and inserting it directly into the GC injection port at a temperature of 220^0 C for desorption of analytes from the fiber. This is the same method that has been successfully applied to GHB. GC data was collected on an Agilent gas chromatograph model 6890 coupled with an Agilent mass selective detector model 5973. The detection of GHV was successful in solutions of water and ethanol. However, problems were encountered with the detection of GVL due to the solvent delay employed to account for the presence of derivatizing agent. A method for the detection of both GHV and GVL simultaneously using liquid chromatography-mass spectrometry (LC-MS) has the potential to solve this problem as derivatization would not be necessary. This is discussed in the scientific session "Comparative Analysis of GHB and GHV II"

GHB, GHV, GVL

K19 Comparative Analysis of GHB and GHV II

Jennifer Wiseman Mercer, BS; Lucy S. Oldfield, MS; Diaa M. Shakleya, PhD; and Suzanne C. Bell, PhD, Bennett Department of Chemistry, West Virginia University, 217 Clark Hall, Morgantown, WV 26506; and Patrick S. Callery, PhD, School of Pharmacy, West Virginia University, Morgantown, WV 26506*

After attending this presentation, attendees will learn about an emerging drug threat, gamma-hydroxy valerate (4-hydroxypentanoic acid, GHV), and a rapid, sensitive, quantitative confirmatory technique for the simultaneous analysis of GHV and its analog gamma-valerolactone (GVL). In the body, GVL and PD are metabolized to GHV with the associated effects. In addition, the synthesis of GHV from gamma-valerolactone (GVL) will be discussed, as GHV is not available for purchase from classic chemical retailers. This presentation will focus on comparative analysis via liquid chromatography with ultraviolet detection (LC-UV) and gas chromatography with mass spectometric detection (GC-MS) and is meant to complement the related presentation entitled "Comparative Analysis of GHB and GHV I."

GHV has been shown to have effects similar to GHB but requires a higher dosage increasing the threat of toxicity and lethality. GHV is anecdotally reported to have a longer duration of action. Given that GHB and its precursors are controlled, drug abusers may switch to GHV. A recent comprehensive internet search revealed that commercial GHV products are sold on many websites. In addition, the effects of GHV also make it suitable for use in drug-facilitated sexual assault. The forensic community needs to be aware of this new drug and prepare to combat its use.

Gamma-hydroxy valerate (GHV) is the 4-methyl-substituted form of gamma-hydroxy butyrate (GHB). As GHV is not available as an analytical standard, a synthesis was developed which involves the hydrolysis of GVL. Thus, the similarities between the two should be exploited to formulate accurate and sensitive confirmatory tests for GHV and its precursors. Liquid chromatography with ultraviolet detection (LC-UV) is a suitable technique for the separation and confirmation of GHB and its analogs. Gas chromatography analysis is more difficult than liquid chromatography because generally GHB must be extracted from complicated matrices and subjected to derivatization due to the small size of the molecule. Thus, developed methods for GC-MS (with *in situ* derivatization) and LC-UV will be compared to determine the advantages and disadvantages of each method.

Since GHB and GHV are small polar molecules, LC provides a more desirable analysis than the more commonly used GC technique. Suspect solutions may be injected directly onto the column, thereby eliminating the need for extraction and derivatization steps required for GC analysis. The LC used in this study was a Shimadzu with the following components: SCL-10A controller, SPD-10A UV-Vis detector, SIL-10AD auto injector, LC-10AD liquid chromatograph (2), DGU-14A degasser, and CTO-10AS column oven. The software used was EZStart 7.2.1. The column used was a µBondapak™ C18 3.9 x 300 mm column. The method is made quantitative by the addition of (S)-(+)-carvone as an internal standard. Data obtained from LC analysis is shown in **Table 1**.

Table 1 – LC Results

Analyte	LOD	LOQ	Range
GHB	5 ppm	50 ppm	50 – 2000 ppm
GHV	5 ppm	50 ppm	50 – 2700 ppm
GBL	100 ppm	200 ppm	200 – 2700 ppm
GVL	25 ppm	100 ppm	100 – 2700 ppm

The GC was an Agilent model 6890 coupled with an Agilent mass selective detector model 5973. An HP-5 capillary column (30 m x 0.25 mm i.d, 0.25 µm film thickness) was used for chromatographic analysis. The method was made quantitative by the use of 1,5-pentanediol as an internal standard. Data obtained from GC analysis is shown in **Table 2**. Future studies will test the methods' suitability for beverage and urine analysis.

Table 2 – GC Results				
Analyte	LOD	LOQ	Range	Quantitative Ions
GHB	0.1 ppm	5 ppm	5 – 100 ppm	233
GHV	0.1 ppm	5 ppm	5 – 100 ppm	75

GHV, GHB, LC-MS

K20 Analysis of THC and Its Metabolites Utilizing LC/MS/MS

Tania A. Sasaki, PhD, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404; and Dea Boehme, Keith Nakagawa, and Fabiola Nunes-Daniel, Ventura County Sheriff's Crime Lab, 800 South Victoria Avenue, Ventura, CA 93009*

After attending this presentation, attendees will understand the utility of LC/MS/MS for toxicological analyses. Analysis of THC and its metabolites will be presented, but the basic principles can be extended to other analytes of interest.

This presentation will impact the forensic community and/or humanity by demonstrating a simple, sensitive technique for analysis of THC and its metabolites in biological matrices. Sample preparation and analysis times are significantly reduced versus other techniques. This presentation will also give the community more exposure to LC/MS/MS, which can be used as a complementary technique to GC/MS.

Cannabis (marijuana) is the most commonly used illicit drug. Δ^9-Tetrahydrocannabinol (THC) is the active compound in cannabis and its major metabolites are 11-hydroxy- Δ^9-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy- Δ^9-tetrahydrocannabinol (THC-COOH). Because of its prevalent use, there is an increased demand for detection and quantification of THC and its metabolites in toxicological assays. Until recently, screening has been accomplished by immunoassay and quantification utilizing GC/MS. Over the past 10 years, LC/MS/MS use has significantly increased in many analytical areas, including toxicology. LC/MS/MS often achieves better detection limits versus GC/MS and sample preparation is less labor intensive. A quick and rugged method for analysis of THC and its major metabolites was developed using a hybrid triple quadrupole/linear ion trap LC/MS/MS system. This instrument has the capability to acquire qualitative and quantitative data in a single experiment.

Sample preparation consisted of simple protein precipitation or solvent extraction followed by centrifugation. Detection limits of less than 0.1 ng/mL for all analytes were obtained with precision and accuracy within 10% and 5%, respectively. The reproducibility and ruggedness was shown to be extremely good.

An LC/MS/MS technique for extraction, detection, and quantification of THC and its metabolites was developed. This technique showed excellent precision and accuracy and improved detection limits versus GC/MS. Sample preparation was also greatly simplified versus GC/MS analysis, especially since no derivatization was required. Run times were less than 10 minutes, which further reduced the overall analysis time. The ability to acquire both qualitative and quantitative data in a single assay allowed for detection, confirmation, and quantification in a single run.

LC/MS, THC, Toxicology

K21 Simultaneous Analysis of Thebaine, 6-MAM and 6 Abused Opiates in Postmortem Fluids and Tissues Using Zymark® Automated Solid-Phase Extraction and Gas Chromatography-Mass Spectrometry

Robert D. Johnson, PhD, and Russell J. Lewis, PhD, Federal Aviation Administration, AAM-610, CAMI Building, Room 205, 6500 South MacArthur Boulevard, Oklahoma City, OK 73169*

After attending this presentation, attendees will gain knowledge in potentially differentiating between opiates derived from ingested poppy seeds and opiates taken as medication.

This presentation will impact the forensic community and/or humanity by assisting in the potential prevention of false opiate positives due to poppy seed consumption.

Opiates are some of the most widely prescribed drugs in America and are often abused. Demonstrating the presence or absence of opiate compounds in postmortem fluids and/or tissues derived from fatal civil aviation accidents can have serious legal consequences and may help determine the cause of impairment and/or death. However, the consumption of poppy seed products can result in a positive opiate drug test. A simple method for the simultaneous determination of 8 opiate compounds from one extraction was developed. These compounds are hydrocodone, dihydrocodeine, codeine, oxycodone, hydromorphone, 6-monoacetylmorphine, morphine, and thebaine. The inclusion of thebaine is notable as it is an indicator of poppy seed consumption and may help explain morphine/codeine positives in cases where no opiate use was indicated. It must be stressed, however, that it is possible following poppy seed ingestion to find morphine and codeine in urine without detecting thebaine. Therefore, the absence of thebaine cannot preclude poppy seed consumption as the source of morphine and codeine present in a case.

Specimens types analyzed during this study were blood, urine, liver, kidney, and skeletal muscle. Three mL aliquots of liquid specimens and 1 g aliquots of homogenized tissue specimens were precipitated with acetonitrile and extracted using a common solid phase extraction (SPE) procedure in combination with an automated SPE system. This method incorporated gas chromatography/mass spectrometry, and trimethyl silane (TMS) and oxime-TMS derivatives. The limits of detection ranged from 0.78 – 12.5 ng/mL. The linear dynamic range for most analytes was 6.25 – 1600 ng/mL. The extraction efficiencies ranged from 70 – 103%. Accuracy, measured as the relative error obtained from the concentration obtained from a control versus its target value, ranged from 1-14% and precision, measured as the relative standard deviation obtained from 5 repeated injections of the same control, ranged from 1-9%. This method was applied to 8 separate aviation fatalities where opiate compounds had previously been detected. The analytical results obtained will be presented.

Poppy Seeds, Thebaine, GC/MS

K22 A Novel LC/MS Method for the Quantitation of Vardenafil

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After attending this presentation, attendees will learn an accurate and reliable method for the detection of vardenafil.

This presentation will impact the forensic community and/or humanity by providing the forensic community with an accurate and reliable method for the detection of vardenafil.

Vardenafil is an oral medication used for the treatment of erectile dysfunction. Vardenafil, when used properly, is relatively safe. However, vardenafil has been shown to potentiate the hypotensive effects of nitrates commonly employed in the treatment of certain heart conditions. Moreover, while vardenafil inhibits phosphodiesterase type 5 enzyme, it also has a high affinity for phosphodiesterase type 6 (PDE6), which is a retinal enzyme involved in phototransduction. The inhibition of PDE6 can result in the inability to discriminate between blue and green colors, resulting in a condition known as "blue tinge." This blue-green impairment could cause problems in the execution of certain tasks. For example, this impairment could lead to a problematic situation for a pilot relying upon instruments during night flights or adverse conditions. During the investigation of aviation accidents, postmortem specimens from accident victims are submitted to the Federal Aviation Administration's Civil Aerospace Medical Institute (CAMI) for toxicological analysis. As new medications are introduced to the market and are subsequently used by aviation accident victims, CAMI's forensic toxicology laboratory is tasked with developing analytical methods for the determination of these compounds. This report presents a rapid and reliable method for the identification and quantitation of vardenafil in biological specimens using LC/MS/MS and MS/MS/MS. This procedure utilizes sildenafil-d_8, which is closely related to vardenafil, as an internal standard for more accurate and reliable quantitation. This method incorporates solid-phase extraction (SPE) and LC/MS/MS and MS/MS/MS utilizing an atmospheric pressure chemical ionization ion trap mass spectrometer in the positive chemical ionization mode. Using a common basic drug SPE procedure, the extraction recoveries for blood controls at 2, 20, and 200 ng/mL ranged from 94 – 97%. The limit of detection for vardenafil was determined to be 0.19 ng/mL. The linear dynamic range for this compound was 0.39 – 200 ng/mL. This novel analytical procedure proved to be simple, accurate, and robust for the identification and quantitation of vardenafil in biological specimens.

Vardenafil, LC/MS/MS, Aviation Death Investigation

K23 Oxycodone and Oxymorphone Glucuronide Conjuagtes in Urine From Pain Management Patients

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Oxycodone is generally present in urine as readily extractable free, unconjugated form; however, its oxymorphone metabolite is extensively conjugated. The goal of this presentation therefore, is to assure detection or extend the detection time for oxycodone, urine should be hydrolyzed prior to analysis.

This presentation will impact the forensic community and/or humanity by assisting forensic toxicologist in the detection of oxycodone and its oxymorphone metabolite in urine specimens and in evaluation of oxycodone urine concentrations.

The concentrations of free and glucuronide conjugates oxycodone and its oxymorphone metabolite in urine specimens collected from 200 different pain management patients will be presented. These patients were receiving oxycodone as the sole opiate derivative used for pain control either in combination with acetaminophen or in a sustained release dosage form. Daily doses ranged from 20 to 80 mg of oxycodone. Urine specimens were analyzed initially fluorescence polarization opiate immunoassay (FPIA) at a cut-off of 100 ng/mL and oxycodone enzyme immunoassay (EIA) at a cut-off of 100 ng/mL before and after enzymatic hydrolysis with beta-glucuronidase. Following the addition of hydroxylamine, oxycodone and oxymorphone oxime derivatives were isolated from urine by solid phase extraction in a Detectabuse™ Gravity GV-65 column as described by the manufacturer (Biochemical Diagnostics Corp.). Oxycodone and oxymorphone oximes were derivatized by the addition of BSTFA [N,O-bis (trimethylsilyl)trifluoroacetamide]. The residues were analyzed on a Hewlett Packard (Palo Alto, CA) 6890 gas chromatograph with a split/splitless injection port, a 7673 auto-sampler and a 5973A mass selective detector (MSD). The column was an HP-5 capillary column (5.0 m x 0.1 mm id x 0.40 um film thickness). Column flow rate was 1.0 mL/min; inlet pressure at 60.53 psi; and injection was in the 4:1 split mode with a split flow of 4.0 mL/min. The oven temperature program was: initial 170^0C for 0 min., then ramped at 30^0C/min. to 280^0C that was held for 0.33 min. Under these conditions the retention times in minutes of TMS derivatives were oxycodone-oxime, 3.32; and oxymorphone-oxime, 3.62. The MSD was operated in the SIM mode using the following ions: oxycodone-oxime-TMS, 459, 444 and 368; oxymorphone-oxime-TMS, 517, 502 and 412; $^2H_3$-oxycodone-oxime-TMS 462 and 447; and $^2H_3$-oxymorphone-oxime-TMS, 520 and 503. Oxycodone and oxymorphone were quantitated using a single point calibrator of each at 500 ng/mL with internal standards of the respective deuterated analogs. The assay was linear from 50 to 10,000 ng/mL of oxycodone and oxymorphone. The lower limit of quantification of each analyte was 100 ng/mL. In the 200 unhydrolyzed urine specimens, the mean oxycodone concentration was 2,450 ng/mL (range, 119-7,600 ng/mL) and the mean oxymorphone concentration was 131 ng/mL (50-1,000 ng/mL). Following glucuronidase hydrolysis of these specimens, the mean oxycodone concentration was 4,000 ng/mL (range, 149-18,600 ng/mL) and the mean oxymorphone concentration was 2,900 ng/mL (range, 172-54,000 ng/mL). The mean percent of oxycodone and oxymorphone as glucuronides in the urine specimens were 29% (range, <1-85%) and 95.5% (range, 66-99%), respectively. These data demonstrate that oxycodone is generally present in urine as the readily extractable free, unconjugated form; however, its oxymorphone metabolite is extensively conjugated. Therefore, to assure detection or extend the detection time for oxycodone, urine should be hydrolyzed prior to analysis.

Oxycodone, Glucuronides, Oxymorphone

K24 A Study of the Adulteration of Chinese Herbal/Patent Medicines Collected From New York City's Chinatown With Western Pharmaceuticals

Richard A. Stripp, PhD, and Gretchen Miller, BS, John Jay College of Criminal Justice-The City University of New York, 899 10th Ave., New York, NY 10019*

After attending this presentation, attendees will understand the dangers associated with consumption of some unregulated patent/herbal medications and the extent to which they contain western drugs.

This presentation will impact the forensic community and/or humanity by making the community aware of the potential exposure of individuals to western drugs upon consumption of some imported herbal preparations.

In America, recent growth in the popularity of Chinese Herbal/Patent Medicines (CHM/CPM) has generated concerns as to the safety of these and other herbal remedies. These agents are consumed due to their suggested ability to aid with many maladies ranging from hypertension, cold and flu, inflammation, chronic and acute pain, asthma, arthritis, sexual dysfunction, stress and anxiety, to name but a few. Lack of strict federal regulations has lead to the possibility of adulteration of these products with western drugs or other chemical contaminants. This may pose a problem among consumers unaware that they may contain hazardous ingredients. Additionally, potentially serious drug interactions are also possible due to the lack of proper labeling of these products. In order to determine the extent of adulteration and/or mislabeling of CHM/CPM in New York City's Chinatown, the laboratory has conducted a study to screen these products for mislabeled or undeclared pharmaceuticals and therapeutic substances. Representative samples in the form of pills, tablets, creams and teas were screened and confirmed by appropriate analytical techniques including, thin layer chromatography (TLC), gas-chromatography mass spectrometry (GC/MS), and high performance liquid chromatography (HPLC). To date, approximately 25% of all samples analyzed contained western pharmaceuticals, undeclared or mislabeled substances. Drugs that have been identified in CMH/CPM thus far will be presented and include; promethazine, chlorpheniramine, diclofenac, chlordiazepam, reserpine and steroidal anti-inflammatory drugs.

Herbal Medicines, Adulteration, Western Pharmaceuticals

K25 Comparison of the Intercept® and Salivette® Oral Fluid Specimen Collection Devices for the Detection of Marijuana Use

Richard Crooks, PhD, James J. Borowski, BS, and Lisa D. Tarnai, MS, Scientific Testing Laboratories, Inc., 450 Southlake Boulevard, Richmond, VA 23236; Cheng I Lin, PhD, Lin-Zhi International, Inc., 687 North Pastoria Avenue, Sunnyvale, CA 94085; and Alphonse Poklis, PhD, Virginia Commonwealth University School of Medicine, Box 98-0165 MCV/VCU Station, Richmond, VA 23238-0165*

The goal of this presentation is to inform forensic toxicologists concerning the analytical efficiency of two oral fluid collection devices for detection of marijuana constituents and their utility for detection of marijuana use as compared to urine specimens.

This presentation will impact the forensic community and/or humanity by improving understanding of oral fluid testing and detection of marijuana use.

A comparison of the analytical efficiency of two different oral fluid collection devices and immunoassay systems for detection of marijuana constituents; and the detection of marijuana use by these oral fluid methods as compared to urine drug testing will be presented. Oral fluid specimens were collected by both the Intercept (OraSure Technologies, Inc.) and the Salivette (Sarstedt) collection devices and a urine specimen was obtained from 519 suspected marijuana users. The oral fluid specimens collected by the Intercept device were initially analyzed by the Cannabinoids Intercept Micro-Plate Elisa Immunoassay (OraSure) in a Personal Lab (Trinity Biotech) at a cut-off tetrahydrocannabinol (THC) concentration of 1 ng/mL. The oral fluid specimens collected by the Salivette device were initially analyzed by the Oral Fluid Cannabinoid Enzyme Immunoassay (Lin-Zhi International, Inc.) in a Hitachi 717 modified for sample volume of 60 uL at a cut-off THC concentration of 5 ng/mL. Urine specimens were initially analyzed by the Emit II Plus Cannabinoid Enzyme Immunoassay (Syva Co.) in a Hitachi 717 at a cut-off tetrahydrocannabinolic acid (THCA) concentration of 50 ng/mL. Any positive initial test was confirmed by GC/MS. THC was isolated from oral fluid by liquid/liquid extraction and derivatized with MSTFA. TMS-THC was identified by monitoring 386, 303, 387 m/z ions. The LOQ of the method was 0.2 ng/mL. . THCA was isolated from urine by liquid/liquid extraction and derivatized with MSTFA. TMS-THCA was identified by monitoring 371, 473, 488 m/z ions. The LOQ of the method was 3.0 ng/mL. Approximately, 10% (51/519) of at least one of the specimens from the donors was positive for THC or THCA by GC/MS analysis; THC was detected in 32 oral fluid and THCA in 51 urine specimens. There was complete concordance between positive THC findings by both immunoassays and GC/MS for only 23 of the 32 oral fluids specimens. However, there was a 97% concordance between negative THC findings by both immunoassays and GC/MS for 477 of the 491 oral fluids specimens. Comparing results from the Intercept method with GC/MS, the analytical sensitivity of the Intercept/Elisa method was 94% and the analytical selectivity was 99.4%. Comparing results from the Salivette method with GC/MS, the analytical sensitivity of the Salivette/Enzyme immunoassay method was 78% and the analytical selectivity was 99.8%. The difference in sensitivity between these methods was due to the difference between the cut-off values; Elisa, 1 ng/mL and enzyme immunoassay, 5 ng/mL. The Salivette/Enzyme immunoassay method yielded 7 false negative results. The Intercept/Elisa method was less selective than the Salivette/Enzyme immunoassay as it yielded 3 false positive results. Urine testing resulted in a significant increase in positive cannabinoid findings as compared to oral fluid testing. While oral fluid testing yielded only 32 positive findings, 51 urine specimens tested positive by initial screening and GC/MS. This represents a 68% increase in the detection of marijuana use. This increase was due to the prolonged excretion of THCA in urine as compared to the presence of THC in oral fluid. The presented data demonstrates the need for very low THC cut-off concentrations (1 ng/mL) when screening oral fluids for consistent detection of cannabinoids. Additionally, marijuana use is best detected by urine drug testing, even at a THCA cut-off value of 50 ng/mL, as compared to oral fluid testing at 1 ng/mL.

Oral Fluids Testing, Urine Drug Testing, Cannabinoids

K26 The Detection of THC and THCA in Whole Blood Using Two-Dimensional Gas Chromatography and EI-MS

Rodger D. Scurlock, PhD, Greg B. Ohlson, MS, and David K. Worthen, BA, Arizona Department of Public Safety, Central Regional Crime Lab, 2102 W. Encanto Blvd, Phoenix, AZ 85005*

After attending this presentation, attendees will learn about an improved method for measuring THC and THCA in blood by GCMS. The new method includes the easy use of 2-dimensional chromatography to greatly reduce matrix interference and improve the limit-of-detection.

This presentation will impact the forensic community and/or humanity by demonstrating an easy and inexpensive improvement for the determination of cannabinoids in whole blood by GCMS .

A method is described for the simultaneous analysis of THC and its carboxylic acid metabolite, THCA as their TMS derivatives using 2-dimensional chromatography and EI-MS detection, (2-D GCMS). The addition of a Deans switch to a standard GC oven allows the use of two chromatographic columns of differing stationary phase to greatly reduce matrix interference. The Limit of Quantitation (LOQ) for THC and THCA was determined to be 1.0 ng/ml. The between-run precision at 1.0 ng/ml (N=25) was 7.7 and 11.1 % for THC and THCA, respectively. The method is linear from 1 to 100 ng/mL.

Sample Preparation: Internal standard was added (10 ng of THC-d3 and THCA-d3 in methanol) to 1.0 mL of whole blood. To each sample 2.0 mL of cold (-20° C) acetonitrile was added and immediately vortexed for 30 seconds before proceeding to the next sample. (The cold acetonitrile produces a more finely divided protein precipitate than did room-temp acetonitrile.) The samples were briefly centrifuged, the supernate was decanted to a clean tube and the solids were discarded. 2.0 ml of DI water was added to each sample before it was poured onto the SPE column (*Cerex*

polychrome THC columns from *SPEware*). The columns were washed with a 1.0 ml mix of water/acetonitrile/NH_4OH (85/15/1, prepared daily) and dried for 10 minutes by a flow of nitrogen. The THC was eluted into a tube with 2.0 ml of ethyl acetate followed by 10 minutes of drying. The THCA was eluted into the same tube with 2.0 ml of a hexane/ethyl acetate/acetic acid mix (90/10/3, prepared daily). All flows through the column were ~1 drop/sec controlled by positive pressure. The samples were evaporated to dryness at 50° C under a stream of nitrogen. 50 uL BSTFA+1%TMCS and 50 uL ethyl acetate were added to each tube, the tube was vortexed and the contents were transferred to a GCMS vial. The vials were crimp-capped and heated at 70° C for 20 minutes.

Instrumentation: GCMS-FID: The gas chromatograph was an Agilent 6890 equipped with a FID and a Deans switch. The Deans switch, from Agilent Technologies, consists of a second EPC (electronic pressure control) module, a solenoid switch that is outside the oven and a manifold inside the oven to connect the GC columns. The Mass Spectrum detector was an Agilent 5973. The carrier gas was helium. The injection port temperature was 250° C and the transferline was at 300° C. The MSD was operated at 200 volts above the tune. The SIM ions were: m/z 386.3, 371.3, 303.3 for THC, m/z 389.3, 374.3 for THC-d3, *m/z* 371.3, 473.3, 488.3 for THCA and m/z 374.3, 476.3 for THCA-d3. The dwell time for all ions was 25 milliseconds and "high" resolution was selected. The ion mass was determined by scanning each SIM ion in 0.1 *m/z* units. The oven program was: initial temp 120^oC, increased at 20°/min to 200^oC, further increased at 10°/min to 250° C, increased again at 25°/min to 300° C and held for 0.5 min. The injection port liner was a deactivated 4mm splitless gooseneck with glass wool from Restek. The injection volume was 2 uL.

Deans Switch Parameters: Column 1 was a RTX-200 (20m, 1.18mm id, 0.20 um df). Column 2 was a DB-17 (15m, 0.25 mm id, 0.25 df). The pressure for the injection port and the Deans switch were calculated using the Deans Switch Calculator software (Agilent Technologies) to achieve 1 ml/min flow through the primary column and 2.0 ml flow through the secondary column at an oven temperature of 200° C. The injection port was set for constant pressure at 41.06 psi and the Deans switch pressure was 16.98 psi. The post-run program was set for 1 minute with the oven temp at 320^oC and the inlet pressure at 1 psi. With the inlet pressure at 1 psi in the post-run, the carrier gas flow through the primary column is reversed. This ability to back-flush the primary column is an advantage of the Deans switch system and reduces the maintenance frequency of the primary column. The secondary column stays remarkably clean because only a small fraction of the injection volume for each chromatographic run flows through it.

Marijuana, Blood, GCMS

K27 Combined Drug and Alcohol Use in Drivers Suspected of Vehicular Assault and Homicide

Barry K. Logan, PhD, Washington State Toxicology Laboratory, Washington State Patrol Forensic Laboratory Services Bureau, 2203 Airport Way South, Seattle, WA 98134; and Laurie Barnes, BS, JD, Washington State Toxicology Laboratory, Washington State Patrol Forensic Laboratory Services Bureau, 2203 Airport Way South, Seattle, WA 98134*

After attending this presentation, the attendee will understand the limitations of current toxicological practices in identifying drug impairment by drivers involved in serious injury traffic accidents; recognize the major drug classes known to be involved, and the degree of combined alcohol and drug involvement.

This presentation will impact the forensic community and/or humanity by attempting to be the first to document the combined use of drug and alcohol use in drivers suspected of vehicular homicide and assault. This should improve practices of traffic law enforcement, provide a basis for allocation of enforcement assets to detecting symptoms of drug impairment, and encourage comprehensive drug testing in alcohol DUI cases.

Since the relationship between blood drug concentrations and the degree of effects associated with those is not well established for most drugs, investigation of vehicular assault and homicide cases should ideally include an assessment of the suspect's degree of sobriety by a trained officer proximate to the time of the accident. This is often not possible due to injuries sustained by the subject. In those cases when a blood sample is collected it falls to the forensic toxicologist to interpret those findings, and relate them to the suspects known driving behavior. Current practice in most jurisdictions however, driven by limited toxicological resources, is that if a suspect's blood alcohol exceeds the legal limit, no drug testing is performed and the subject is prosecuted based on the alcohol result and its known effects.

In an effort to assess the true rate of drug use and combined alcohol and drug use in the impaired driving population, samples taken from suspects in vehicular assault and homicide cases were subjected to comprehensive drug testing, irrespective of the blood alcohol concentration.

From a review of cases received during 2002 and 2003, 804 cases were identified where the driver was considered a suspect in a vehicular assault or homicide case. Of these, 700 were available in sufficient quantity for comprehensive testing for priority drug classes by immunoassay (EMIT for barbiturates, benzodiazepines, cannabinoids, cocaine metabolite, methadone, opiates, phencyclidine, propoxyphene and tricyclic antidepressants), alcohol, and basic drugs by gas chromatography. Since this was an assessment of incidence of drug use, determinations were qualitative only.

Table 1 shows the relative frequency of alcohol and drug use alone and in combination. Alcohol positive cases were those with blood alcohol concentrations 0.01g/100mL and greater. Drug positive cases were those with one or more drugs capable of causing impairment.

Table 1. Drug and alcohol positivity rates for all cases (n=700)

	Drug Positive	Drug Negative	Totals
Alcohol Positive	235 (33.5%)	223 (31.8%)	458 (65.4%)
Alcohol Negative	115 (16.4%)	126 (18.0%)	242 (34.5%)
Totals	351 (50.1%)	349 (49.9%)	700

Of the 700 cases tested, 126(18.0%) had no detectable alcohol or drugs. There may be a variety of reasons why samples were submitted in these cases. It would include the fact that some agencies have a policy of submitting samples from the driver in any serious injury accident, whether or not there is evidence of fault, or of drug or alcohol use. It would also include individuals who submitted samples to protect themselves in case of civil litigation resulting from the collision. Additionally, drugs such as gabapentin, GHB, lorazepam, and clonazepam, are not detected in the test batteries used in this study. Drugs which are generally not considered to have any significant effect on driving such as caffeine, nicotine, lidocaine, bupivacaine, venlafaxine, citalopram, and other SSRIs were not included in the totals for drug positive cases.

The mean ($\pm$SD) blood alcohol concentration (BAC) among the alcohol positive cases was 0.15 ($\pm$ 0.07) g/100mL (median 0.15, range 0.01 – 0.44g/100mL). This is similar to the average blood alcohol concentration seen in DUI arrests in Washington State, where there is either no collision, or no serious injury.

Of the alcohol positive cases, 235 (51.3%) additionally had drugs which could have contributed to impairment. This is a significant finding since in many jurisdictions a positive blood alcohol result would preclude any further testing for drugs, meaning that the subject's drug use would go undetected. This is important for a number of reasons, including the ability

to fully prosecute the case, negotiations on a plea agreement, assessment or treatment for drug use, and allocation of law enforcement resources.

The data were also examined to assess relative rates of drug use among drivers with high BAC's compared to lower BAC's, and the data are presented in table 2.

Table 2. Drug positivity with respect to blood alcohol concentration.

	Drug Positive	Drug Negative	Totals
Alcohol ≥0.08g/100mL	192 (41.9%)	200 (43.7%)	392 (86.0%)
Alcohol 0.01–0.079g/100mL	43 (9.3%)	23 (5.0%)	66 (14.0%)
Totals	235 (51.3%)	223 (48.7%)	458

Of the alcohol positive cases 392 (86%) had alcohol concentrations of 0.08g/100mL or above. Among these high BAC drivers, 48.9% were positive for drugs. For the low BAC drivers (0.01 – 0.079g/100mL), 65% were positive for drugs. The combination of low levels of alcohol with other drugs having CNS depressant properties such as marijuana, benzodiazepines, opiates and muscle relaxants, can cause impairment in a synergistic manner. When circumstances suggest poor or inattentive driving, and the blood alcohol is less than 0.08g/100mL, further assessment of the drivers sobriety, and collection of a blood sample should be standard procedure.

The data were examined for evidence of any relationship between the drug classes detected as a function of BAC, and the data are shown in Table 3.

Table 3. Relative frequency of major drugs/classes identified as a function of blood alcohol concentration†.

	Alcohol Negative (n=242)	Alcohol 0.01 -0.079g/ 100mL (n=43)	Alcohol ≥0.08g/100mL (n=392)
Any impairing drug	47.5%	65.1%	48.9%
Cannabinoids	9.9%	58.0%	26.7%
Amphetamines	14.9%	6.9%	2.0%
Cocaine	2.4%	6.9%	4.8%
Opiates*	8.7%	27.9%	12.8%
Benzodiazepines*	4.1%	20.9%	8.9%

* Note: Opiates and benzodiazepines may be administered during emergency medical treatment prior to the collection of a forensic blood sample, therefore opiate and benzodiazepine rates should be interpreted with caution in this population.

† Columns will not total to 100% since many subjects were positive for more than one class of drugs.

Overall rates of drug positivity were high in all three groups. Therapeutic drug use was highest in the alcohol negative group with muscle relaxants, antiseizure medications, sleep aids and over the counter drugs being more frequently encountered than in the alcohol positive groups. These drugs can nevertheless cause driving impairment even when used according to directions. Rates of combined marijuana and alcohol use were dramatically higher in the alcohol positive groups, with cannabinoids being detected in 58% of low BAC drivers and 26.7% of high BAC drivers. Combined alcohol and marijuana use, particularly in young drivers has been demonstrated to cause synergistic impairment. Amphetamine use (principally methamphetamine) was highest among the alcohol negative drivers. Other studies have demonstrated relatively low rates of concomitant alcohol use among methamphetamine users.

In conclusion, this study documents the high frequency of combined drug and alcohol use among drivers suspected of impaired driving leading to vehicular assault or vehicular homicide. According to current practice, suspicion of drug use increases when a subject's low BAC is not consistent with their observed impairment, and these data validate that practice. In addition however, the data demonstrate that drug use is a factor throughout the range of BAC, and that investigators should be alert for indicia of drug use in any contact with a suspected alcohol impaired driver.

Toxicology, Impaired Driving, Drugs of Abuse

K28 She "Lost that Lovin' Feelin' " in the Arizona Dust: Angry Teen on Alcohol, Cannabis and Cocaine

Michelle A. Spirk, MS, Arizona Department of Public Safety, Central Regional Crime Laboratory, 2323 North 22nd Avenue, Phoenix, AZ 85009*

After attending this presentation, attendees will be exposed to a Case Study involving interpretive toxicology and drug impaired driving. This case provides valuable discussion material as it lacks many of the factors that would make for an "ideal" DUID case; thus forcing a discussion of how real-life drugged driving interpretations may be handled. Drug effects, DRE evidence, analytical issues, case strengths, weaknesses and summary statements will be covered.

This presentation will impact the forensic community and/or humanity by addressing the difficult challenges associated with drugged driving interpretations. Although many forensic toxicologists are asked to provide these interpretations and related expert testimony, they are rarely presented to colleagues in this type of case study format. Utilizing a detailed Case Study model as presented here, is an excellent way to share knowledge and promote discussion regarding challenging drugged driving interpretations.

Ideal drug-impaired driving cases include significant driving behavior, a DRE evaluation, psychoactive parent drug(s) quantitated in blood obtained close to the time of driving that corroborate the DRE opinion, and driver admissions supporting impairment. This case was *not* ideal as it lacked much of the information allowing an interpretive analysis of driving impairment; thus, it *was* a typical DUID case. A 16-year-old female presented with qualitative blood confirmations of Benzoylecgonine (BE), Carboxy-THC and a low alcohol concentration (0.015g/100mL), obtained two hours and 32 minutes after significant driving behavior resulting in a fatal collision causing the death of a seven-year-old child in a second vehicle. The collision occurred on the Tohono Oodham Reservation at approximately 9:41 pm as the teen drove with her boyfriend, and his two nephews (ages 3 and 7) in the back. An argument ensued resulting in her holding onto his shirt to restrain him as he tried to exit the moving vehicle; the 3-year-old was crying to leave. She then ran a stop sign, making a left turn into the opposing traffic lane and colliding nearly head-on with the second vehicle. She had the moderate odor of an alcoholic beverage on her breath, slurred speech and red bloodshot eyes. Confirmatory blood cutoffs for Cocaine/BE and THC/C-THC were 50ng/mL and 2ng/mL respectively, thus parent drugs may have gone undetected. Later additional quantitative analysis of Cocaine and THC was precluded by sample size and consideration of non-enzymatic hydrolysis of Cocaine during extended refrigerated storage. Alcohol concentration was too low to provide extrapolation evidence. No DRE was available. *Interpretation*: BE is detectable in blood for ~ 48 hours post ingestion; blood BE indicates that Cocaine was present at the time of, or prior to, the blood draw. Duration of effects for Cocaine is 2-4 hours and is dose dependent. Effects consistent with Cocaine influence were: self-absorbed; inattentive; decreased divided attention and increased risk taking. Cocaethylene, although not tested for, is a possible additional contributor to driving impairment. Carboxy-THC detection in blood is highly dependent upon dose and frequency of use; blood Carboxy-THC indicates that THC was present at the time of, or prior to, the blood draw. Duration of effects for THC is 3-6 hours with some complex divided attention tasks up to 24 hrs. Effects consistent with THC influence were: bloodshot eyes; decreased divided attention; difficultly thinking, problem-solving and processing information; decreased car handling performance; slow reaction times; decreased perceptual functions and significantly greater effects when combined with alcohol. The absence of Cocaine and THC in this case does not rule out their presence at the time of driving or sampling, or potential dysphoric effects of cocaine. Driver is a minor, with limited driving experience, thus drug-impaired driving would likely occur at a reduced threshold than for a more experienced driver. *Strengths*: inattentive, inexperienced driving resulting in a fatal collision; admission of recent prior alcohol use; confirmation of polydrug metabolites in blood that

could only come from prior use of potentially impairing and illicit drugs; documented poor judgment, decreased divided attention and increased risk taking. *Weaknesses*: driving distractions; absence of psychoactive drug(s); absence of DRE; minimal documentation of drug impairment at scene. *Summary*: focus on driver's diminished capacity to operate motor vehicle safely; includes her inability to focus on the complex task of driving and not become overly distracted by quarrels, etc. Her recent use of cocaine, alcohol and probable cannabis greatly increase the likelihood of her being a less competent and safe driver.

Cannabis, Cocaine, Drugged Driving

K29 Integrating a New DUI Toxicology Program – Trials and Tribulations

Fiona J. Couper, PhD, and Rory M. Doyle, MSc, Office of the Chief Medical Examiner, 1910 Massachusetts Avenue, SE, Building 27, Washington, DC 20003*

After attending this presentation, attendees will learn about a more coordinated effort in dealing with DUI cases in their local jurisdictions.

This presentation will impact the forensic community and/or humanity by facilitating improvements in public health and safety by increasing the likelihood of successful detection and prosecution of DUI cases.

This presentation primarily focuses on the recent changes to the DUI toxicology program in Washington, D.C. Although covering only 10 square miles, the District currently encompasses at least five different local and federal law enforcement agencies, two toxicology testing facilities, and two separate prosecuting agencies; all of which are responsible for the investigation, detection and prosecution of DUI cases. Over the past two years, representatives from several of these agencies have sought to improve overall coordination, communication, and training across the various agencies. This has included improvements in coordinated traffic stops, filing of police paperwork, sample submission, alcohol and drug testing protocols, preparation of court cases, coordinated training, and funding sources, in addition to extensive legislative changes.

DUI, Toxicology, Testing

K30 What's New in the Drug Evaluation and Classification Program?

Charles E. Hayes, International Association of Chiefs of Police (IACP), PO Box 4597, Salem, OR 97302

After attending this presentation, attendees will gain a better understanding of the Drug Evaluation and Classification Program's role in identifying drug impaired drivers and learn about the program's expansion and successes. They will also learn about the latest curriculum revisions.

This presentation will impact the forensic community and/or humanity by providing the forensic community with a better understanding of the DEC program successes and how the forensic community is a key player in this international program.

After attending this presentation, attendees will understand the role of the International Association of Chiefs of Police (IACP) and the National Highway Traffic Safety Administration (NHTSA) in coordinating, administering and facilitating the Drug Evaluation and Classification (DEC) Program. They will also learn about the program's expansion and successes, as well as learning about the most recent training curriculum changes that all Drug Recognition Experts (DREs) use in helping to identify persons under the influence of drugs. Toxicology is one of the key components of the DEC program and must be aware of revisions and new elements within the program.

This presentation will also update attendees on the DRE data collection tracking system that incorporates toxicology results from the various labs throughout the country. It will also address the DEC program expansion efforts into other states and how the program is expanding outside of the United States.

Drugs, Driving, Evaluation

K31 Application of Models for the Prediction of Time of Marijuana Exposure From Blood of Drivers Arrested for DUI

Ann Marie Gordon, MA, Jayne E. Clarkson, BS, and Barry K. Logan, PhD, Washington State Toxicology Laboratory, 2203 Airport Way, Seattle, WA 98134*

After attending this presentation, attendees will learn how clinically derived models for time of prediction of marijuana use in DUID cases was applied to data from actual driving under the influence cases. The model based upon THC concentrations, alone, was not predictive but the model based upon THC>carboxy-THC ratios had some predictive value.

This presentation will impact the forensic community and/or humanity by demonstrating how driving under the influence of marijuana is a major concern in traffic safety and forensic toxicologists are frequently asked to render opinions as to the subject's impairment. Since time of use is one of the determining factors used to evaluate impairment, the ability to predict the time of use from blood levels would be helpful. This paper discusses the use of a model presented by Huestis et al from clinical data and its application to DUI casework.

Forensic toxicologists are often asked to interpret THC and carboxy-THC levels detected in the blood of subjects arrested for driving under the influence of drugs (DUID). Marijuana has been shown to impair driving performance for up to 3 hours. Providing a reliable estimate for the time of use would help in the interpretation of such cases. In 1992, Huestis et al presented two mathematical models for the time prediction of marijuana exposure from plasma concentrations of THC and carboxy-THC based upon data obtained from participants in clinical studies. Model I utilized plasma THC concentrations and model II utilized carboxy-THC:THC ratios in plasma.

This study applied these models to blood data derived from DUI arrestees. Unlike the controlled clinical data, there are additional limitations to arrestee data. Blood, not plasma is the sample collected in DUI arrests; plasma:blood correlations are approximately 2:1. Second, validation of when THC was used and over what period of time in the driving population is not possible. Many of the arrested drivers admitted repeated use of marijuana over an extended periods of time. Since, both THC and carboxy-THC accumulate in chronic or repeat users, the variability of predicted time from the models would increase.

Driving under the influence of drugs is a major concern in traffic safety. Washington State had 2787 drivers arrested for DUID in 2004. The most frequent drug finding is THC and/or its major metabolite, carboxy-THC (28 % DUID cases). In 2004, 1135 drivers tested positive for THC and/ carboxy-THC; 668 (59%) had reportable levels of the pharmacologically active parent drug, THC (limit of quantitation is 1 ng/mL). From 1997 to 2004, the mean THC concentration decreased from 9.7 to 5 ng/mL, median 4 ng/mL, mode 2 ng/mL and the carboxy-THC levels were 54 ng/mL, median 40 ng/mL.

For this study, positive THC cases were selected in which a Drug Recognition Evaluation (DRE) was performed. DRE cases were selected because the reports are more complete. Of the 323 THC positive DRE reports obtained, the time blood draw on the blood vials was available in 191 cases. These cases were reviewed for a reliable source as to the time of last smoking, (n= 91). The criteria used included cases where the arresting officer visually saw the driver smoking marijuana, found a warm marijuana cigarette in the stopped vehicle, or cases in which the subject admitted to smoking within one hour of the stop. Cases were excluded when the subject cited an actual time of smoking as they were often confused actual time of day frequently stating use was after the stop. Admissions such as "thirty minutes before I was stopped" were included. In the 91 cases, the THC levels averaged 5.2 ng/mL, median 4 ng/mL, carboxy-THC levels 57.6 ng/mL, median 40 ng/mL.

Model I proved not to be useful. There was little correlation between the time of the stop and the predicted time of use the predicted time was always considerably less than the actual time. Model II proved more pre-

dictive for the time of use. The time of stated or observed use ranged from 50 to 233 minutes, average 131 minutes and median 127 minutes. The ranges were limited by the time required to stop and arrest, perform the DRE exam and obtain the blood draw. The average absolute time difference between the time of stated or observed smoking and time of blood draw was 43 minutes, median 30 minutes, standard deviation, 37 minutes. Further, Model II predicted the time of smoking to be within 30 minutes of the actual time in 50.5% of the cases, within 1 hour 73.6% of the time and within 2 hours 94.3% of the time. In 69% of the cases, the predicted value exceeded the actual time of use.

The data presented here is very promising for the usefulness of Model II in predicting the time of marijuana use but some caution must be used due to the limitations to this data. First, cases were pre-selected for short intervals between the time of smoking and the time of the stop. Second, the manner in which the cases were selected may have excluded more chronic smokers as these subjects may be less likely to answer the questions as to time of use or may purposefully misstate time of use. However, even in light of these limitations, it does appear that the time of use predicted by Model II may be useful when considered along with the context of the overall case, including the observed driving of the subject, the observed impairment of the subject during the physical exams and subject's statements when rendering an opinion as to the impairment of a subject arrested for DUID, marijuana.

46	0.505495	42.5736
21	0.230769	30.3341
19	0.208791	37.28421
4	0.043956	
1	0.010989	

Marijuana, Driving Under the Influence, Time of Driving

K32 Recommendations for Toxicological Investigations of Drug Impaired Driving Cases from the Joint SOFT/AAFS Drugs and Driving Committee and the National Safety Council Committee on Alcohol and Other Drugs

Laurel J. Farrell, BA, Colorado Bureau of Investigation, 690 Kipling Street, Denver, CO 80215*

After attending this presentation, attendees will learn about improved analytical protocols for DUID toxicological specimens.

This presentation will impact the forensic community and/or humanity by increasing standardization of analytical protocols in laboratories supporting the DRE program and increasing the effectiveness of DUID prosecution.

In May 2004, under the auspices of the National Safety Council's Committee on Alcohol and Other Drugs (NSC-COAD), a panel of toxicologists, prosecutors, and law enforcement officers trained as Drug Recognition Experts gathered to identify problems with the current system of prosecuting drug impaired driving cases, from the point of detection through adjudication. The panel in their report of the meeting sites lack of standardization of practices in toxicology laboratories supporting DUID programs as one of the major problems. The toxicology laboratory provides analysis of the biological specimen collected during the investigation. The labs need to test for the most frequently encountered drugs in these cases, and use an appropriate level of sensitivity. Along with comprehensive documentation by the DRE officer, good quality forensic toxicology is an essential part of the prosecution of a DUID case.

The Joint SOFT/AAFS Drugs and Driving Committee and the NSC-COAD were charged with identifying the laboratories performing DRE toxicology and to survey these laboratories to document their current practices. This was accomplished with the help of the International Association of Chiefs of Police (IACP). Once the survey was completed, the panel recommended that guidelines be developed to direct the development of more consistent procedures and protocols.

The presentation today will highlight results of the survey providing data on the scope and sensitivity of testing in practice today. Recommendations for Toxicological Investigations of Drug Impaired Driving Cases are being developed and the current status of these recommendations, to include drugs to be included in analytical protocols and appropriate analytical thresholds in blood and urine, will be provided.

DUID, Lab Survey, Laboratory Guidelines

K33 Ambien® - Drives Like a Dream? Case Studies of Zolpidem Impaired Drivers in Wisconsin

Laura J. Liddicoat, BS, and Patrick M. Harding, BS, Wisconsin State Laboratory of Hygiene, 2601 Agriculture Drive, PO Box 7996, Madison, WI 53707-7996*

After attending this presentation, attendees will gain knowledge of the zolpidem impaired driver.

This presentation will impact the forensic community and/or humanity by demonstrating An increased awareness of the growing problem of driving under the influence of drugs in the US.

Zolpidem is a non-benzodiazepine, sedative hypnotic prescribed for short term treatment of insomnia. It is available in strengths of 5 and 10 mg, with a recommended dose of 10 mg (5 mg in elderly) immediately before bedtime. Patients using the drug are directed to take zolpidem only when able to devote a full eight hours to sleep and are cautioned against operating heavy machinery or motor vehicles. There have been no reports of significant residual effect on memory or actual driving when subjects have been tested the morning after taking a single 10 mg dose.

However, .zolpidem produces severe deficits in psychomotor performance and cognitive abilities when driving is attempted within 5 hours of use. Erratic driving, confusion, dazed appearance, slurred speech, incoordination, poor balance and loss of memory are often used descriptors for the zolpidem-impaired driver. Drugged driving cases in Wisconsin have been steadily increasing over the last five years. Zolpidem cases have mirrored this trend, reaching a peak of 45 cases each in 2003 and 2004. Selected zolpidem-impaired driving cases will be discussed.

Zolpidem, Impaired, Driving

K34 Multiple Drug Intoxication in Impaired Drivers: Polypharmacy Challenges

Sarah Kerrigan, PhD, Sam Houston State University, College of Criminal Justice, Huntsville, TX 77341-2296*

After attending this presentation, attendees will be able to recognize some of the common challenges involved in polypharmacy DUI casework.

This presentation will impact the forensic community and/or humanity by facilitating the comprehension of interpretive limitations faced by toxicologists.

Despite the prevalence of driving under the influence of drugs (DUID), these cases often provide a number of unique challenges compared with alcohol-related DUI. Toxicology results are often interpreted within the context of driving behavior, signs, symptoms and other observations made by law enforcement personnel or witnesses. The quality of this supporting documentation can influence the interpretive strategy, and subsequently, the outcome in a court of law.

Interpretive issues may be further complicated by the presence of multiple drugs in a driver. Combinations of drugs or "polypharmacy" DUI

casework may pose additional challenges from a toxicological standpoint. Practitioners must go beyond the pharmacological classification of additive, synergistic and antagonistic effects when evaluating these cases. Laboratorians may overcome some of these challenges by appropriate choice of specimen, scope of testing and quantitative drug analysis. Although interpretation is rarely based upon quantitative drug results in isolation, quantitation may be particularly useful in polypharmacy casework to determine dominant drug factors or substances that are most likely to be responsible for the impairment. In some circumstances, quantitative analysis may also provide complementary information regarding approximate timeframe of drug use, history of drug use (habituation) or acute vs. chronic drug use based upon parent/metabolite concentration ratios. Caution should be exercised when classifying drug concentrations as sub-therapeutic, therapeutic, toxic or fatal in polypharmacy casework due to overlapping ranges, tolerance to the toxic effects of some drugs in habitual users and additive effects.

A series of ten cases involving drivers who tested positive for multiple drugs will be presented. Driving behavior, signs, symptoms and toxicology results will be discussed for cases involving combinations of central nervous system depressants, stimulants, opioids and cannabinoids. The series highlights some of the common challenges faced in polypharmacy casework such as additive and combination effects caused by drugs within the same or different drug classifications, the value of quantitative drug analysis to determine which drugs are most likely to be responsible for the observed impairment, the value of qualitative drug analysis for certain drugs, and the need for supporting documentation.

Drugs, Driving, Impairment

K35 Importance of Peer-Review and Publication for Admission of Expert Testimony in Civil and Criminal Litigation

Alan W. Jones, PhD, DSc, Department of Forensic Toxicology, University Hospital, Linkoping, 581 85, Sweden*

After attending this presentation, attendees will learn about the purpose, meaning, and proper use of peer-reviewed literature in specific reference to civil and criminal litigation.

Since the proliferation of scientific admissibility rules, e.g., *Daubert*, there has been a significant weight placed on peer-review. The effective use of peer-reviewed literature in the forensic setting can be abused and misused. A clear understanding of what "peer-reviewed" means as well as its relevant application will impact the forensic community and/or humanity by assisting in the proper use of this element within the scientific and legal arenas.

Much has been written about admissibility of expert testimony in the wake of the U.S. Supreme court decision in the case of *Daubert vs. Merrill Dow Pharmaceuticals.* The so-called *Daubert* principles or criteria for admission of expert testimony have gained wide acceptance not only in many U.S. states but also in other countries where an adversarial system of justice operates (e.g. UK and Australia). One of the *Daubert* principles asks whether a theory or technique has been subjected to peer-review and publication. However, publication should not be the *sine qua non* of admissibility. Publication of research is an integral part of the scientific process and a scientist publishes to spread information to colleagues, to gain credit for the work and to enhance his or her reputation. The vast majority of work published in mainstream scientific journals will never ever be used in civil or criminal litigation. On the other hand, many scientific articles are cited and used daily by both the defense and prosecution attorney to bolster their arguments. But does it really matter where a paper is published? Are some journals more reputable than others? How can scientific journals be compared and contrasted? Can peer-review uncover flawed work and/or plagiarism and thus avoid junk science seeing print? Flawed publications results in flawed expert testimony.

Most would agree that a scientific journal is only as good as its peer-reviewers. Peer-review of manuscripts submitted for publication enjoys a 250-year history although the peer-review process has come under attack from several quarters in recent years. Some maintain that the system is outdated and is in urgent need of overhaul. Allegations of bias, nepotism, competing or conflicts of interest have been raised. The advent of web-based journals many of which operate a completely open peer-review system might be something to consider for print-journals. On the web, the entire pre-publication history of an article is available for scrutiny, which is in stark contrast to the traditional "strictly confidential" peer-review reports of paper journals.

With peer-review and publication gaining so such importance in criminal and civil litigation, perhaps the time has come to disclose peer-review reports of manuscripts or make them open to discovery. Most journals operate a single-blind peer-review evaluation with the names of the reviewers not being reveled to the authors of the manuscript. However, some journals request that peer-reviewers now sign their reports and others would like to see the names of these individuals included as an endnote on the published article. Just because an article is published in a peer-review journal does not make the findings or conclusions gospel. What Sir Winston Churchill once said about democracy can be said about peer-review, namely *"it is the worst system in the world but better than all the rest."*

The purpose of expert evidence is to provide the court with information derived from scientific research and studies far removed from the experience, skill and knowledge of a judge and jury. Unlike an ordinary witness who provides factual evidence an expert witness can testify to opinion gleaned from his or her own specialized scientific, technical or medical knowledge. As a result of *Daubert* expert evidence has come under close scrutiny. Some recent high profile cases in UK involving complex and equivocal forensic-medical evidence has led to very serious miscarriages of justice. The backlash from these cases has called into question not only the reliability and admissibility of expert testimony but also the entire adversarial system. The notion of pre-trial hearings and the use of a single joint expert and even jury-free trials might be more appropriate in some cases, especially in civil litigation.

Peer-Review, Publications, Expert Testimony

K36 A Dog, a Lawyer, and a Toxicologist - Proving the Need for Pre-Trial Conferences

Robert A. Middleberg, PhD, National Medical Services, Inc., 3701 Welsh Road, Willow Grove, PA 19095*

After attending this presentation, attendees will understand the need for pre-trial conferences between lawyers and toxicologists is of paramount importance for the successful use of such testimony. Through case example, and without pre-trial conference, demonstration of how toxicological testimony was rendered useless and ineffective, and in fact, detrimental to the attorney's claim, even though he requested the scientific assistance.

This presentation will impact the forensic community and/or humanity by effectively using toxicological testimony in a helpful, time-effective manner.

Toxicological testimony can be of significant importance in both criminal and civil litigation. Toxicologists, unless specifically trained though law school, do not have the requisite knowledge to understand courtroom tactics, policies or procedures; on the other hand, attorneys do not, generally, have the knowledge base to understand the scientific principles behind toxicological analyses and interpretations. The successful melding of the two disciplines is necessary for the successful presentation of toxicological data in legal proceedings. One of the primary means of ensuring successful use of toxicological testimony is through extensive and intensive pre-trial conferences, with the emphasis on the plural. Remarkably, experienced toxicologists consistently testify with the

absence, or cursory forms, of such conferences, albeit not of their own choice. One case in point will demonstrate the ineffective use of a "pre-trial" conference and the ultimate effect on the case outcome. In this case, the attorney failed to recognize the lack of significance of the toxicological data, and subsequent interpretation, in the case, despite thinking the contrary. As a result, the courtroom presentation was farcical in nature, humorous and disastrous.

Pre-Trial Conference, Toxicologist, Lawyer

K37 Evaluation of Analytical Toxicology Test Data in Criminal Prosecutions and Civil Litigations

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After attending this presentation, attendees will understand the significance and benefits of data review for all litigations involving toxicological analyses as well as the potential pitfalls of not having data reviewed.

So often, litigation involving toxicological issues focuses solely on the interpretive aspects of reported analytical results. However, the predicate is that the interpretation is based on good analytical data. Experience has demonstrated that this is not always the case. Thus, the independent review of analytical data is critical before any interpretive issues can be deduced. This presentation will impact the forensic community and/or humanity by highlighting the duality of the forensic toxicologist as both bioanalytical chemist and toxicologist and the importance and necessity of both roles.

The duties and responsibilities of the forensic toxicologist include: qualitative and quantitative analysis of drugs or poisons in biological specimens; and the interpretation of the analytical findings as to the physiological or behavioral effects upon the specimen donor, whether living or at the time of his death. It is most often in the role of interpreter of drug or poison effects that the toxicologist is subjected to adversarial questioning in legal proceedings. Usually, the actual analytical testing that gives rise to the basis of his interpretations is seldom questioned. This assumption of properly performed and documented analytical testing is well justified in certain areas of toxicology such as in the highly regulated urine drug testing industry or in blood and breath alcohol testing in cases of impaired driving. However, in many criminal cases or civil litigations, particularly those involving drugs or poisons not commonly encountered in toxicology testing, it is prudent for attorneys to obtain copies of the analytical testing data for review by an experienced forensic toxicologist. The significance and often surprising revelations of such a review will be highlighted by example cases.

Toxicology, Data, Analysis

K38 Gamma Hydroxybutyrate (GHB)-Related Deaths: Review of 194 Cases

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After this presentation, attendees will understand the nature/range of lethal risks posed by gamma hydroxybutyrate (GHB) and will be familiarized with toxicological/pathological findings on 194 GHB-related deaths. The presentation will raise awareness among law enforcement, clinicians, and the public regarding the lethality of GHB and aid in recognition and confirmation of GHB-related deaths and in harm reduction through public education.

GHB and its analogs, gamma butyrolactone (GBL) and 1,4 butanediol (BD), are drugs of abuse that have been sold as dietary supplements for purported health benefits. GHB/analogs have resulted in overdoses, addiction and lethal withdrawal, and have been used to facilitate drug-facilitated sexual assault (DFSA).

Medical Examiners and coroners across the U.S. and abroad were contacted to request searches for cases of GHB-related deaths and specific cases identified through the Project GHB website and media reports. Toxicology findings were requested and, whenever possible, autopsy reports with investigative summaries. GC/MS cut-offs of 50 mg/L in postmortem blood, 5 mg/L in antemortem blood, and 10 mg/L in urine were used for inclusion of cases.

194 GHB-related deaths were identified from 1995-2005; case identification is incomplete due to non-searchable records and confidentiality restrictions. Decedents included 133 men (69%) and 61 women (31%), ages 15-53 yrs (mean=27.9 yrs). 183 (94.5%) had cardiopulmonary arrest (29 with aspiration or asphyxiation), 6 (3%) drowned in hottubs/bathtubs, 4 (2%) died in lethal motor vehicle collisions, and 1 (0.5%) died of smoke inhalation from a fire started while GHB-intoxicated. One case involved DFSA, as supported by history and pathological findings.

179 had autopsy reports with toxicology, 2 had external exam findings with toxicology, and 13 had toxicology data only. Of 179 with autopsy findings, 150 had pulmonary edema/congestion. 59 had autopsies noting an enlarged heart; of these, 37 had LVH, 1 had RVH, 1 had LVH/RVH, and 20 had cardiomegaly without LVH/RVH noted. Analysis will be done to assess for correlation with chronic GHB use/dependence and histories of use of anabolic steroids, GHB, and other drugs including methamphetamine and cocaine.

Of 194 deaths, 177 had GHB confirmed with blood GHB levels, 16 with urine GHB levels only, and 1 with a chest fluid GHB level. Of 177 deaths with confirmatory blood GHB levels, there were 60 deaths (34%) with GHB as the sole intoxicant, 61 deaths (34.5%) with ≥1 depressant co-intoxicants, 25 (14% of total) with ≥ 1 stimulant co-intoxicant, and 31 (17.5%) with both stimulant and depressant co-intoxicants. See Table 1 for toxicology findings.

K 38 - Table 1. Toxicology findings for 177 deaths confirmed by blood GHB and/or BD levels

	#deaths (%)	# blood samples	Postmortem (PM) GHB mean and range	Antemortem (AM) GHB mean and range	Postmortem (PM) BD mean and range
GHB only	60 (34%)	71 total 69 PM, 2 AM	Mean 560.7 mg/L Median 319 mg/L Range 66-4400 mg/L	Mean 334.5 mg/L Range 159-510 mg/L	Mean 164.5 mg/L Range 7.6-220 mg/L
GHB and ≥1 depr. co-intox	61 (34.5%)	65 61 PM, 4 AM	Mean 442.5 mg/L Median 286 mg/L Range 59-2300 mg/L	Mean 262.2 mg/L Range 17-562 mg/L	None
GHB and ≥1 stim. co-intox	25 (14%)	28 26 PM, 2 AM	Mean 525.3 mg/L Median 346.0 mg/L Range 210-2900 mg/L	Mean 317.0 mg/L Range 190-444 mg/L	None
GHB and stim/depr co-intox	31 (17.5%)	33 31 PM, 2 AM	Mean 455.5 mg/L Median 259 mg/L Range 67-1550 mg/L	Mean 715 mg/L Range 700-730 mg/L	None

An additional 16 deaths were confirmed with urine GHB levels only (mean urine GHB 1544 mg/L, range 67-5950 mg/L). These included 3 deaths (19%) with GHB and no co-intoxicants, 7 deaths (44%) with $\geq$ 1 depressant co-intoxicants, 2 deaths (12%) with $\geq$ 1 stimulant co-intoxicants, and 4 deaths (25%) with both stimulant and depressant co-intoxicants. One death was confirmed with chest fluid GHB levels only (316 mg/L); this death occurred with a depressant co-intoxicant.

GHB concentrations exhibit unpredictable variability between collection sites. Heart/femoral blood ratios ranged from 0.6 to 1.93 in 11 cases. 31 vitreous samples contained an average GHB 280.6 mg/L, range 9-1300 mg/L.

In conclusion, data collection is ongoing and additional analysis will be performed on multi-site sampling data and to investigate correlations between pathologic findings and use patterns. The series demonstrates that GHB may be lethal, even without co-intoxicants, in a variety of ways, and the public, clinicians, and law enforcement must be made aware of these risks.

K39 Postmortem Tissue Distribution of Atomoxetine in Fatal and Non-Fatal Dosing Scenarios – Three Case Reports

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After attending this presentation, attendees will learn at what tissue concentrations to expect to find atomoxetine, and at what blood and tissue levels atomoxetine can be considered non-toxic. This data will be useful, since the presence of atomoxetine is already becoming prevalent in medical examiner cases, as the drug is an increasingly prescribed alternative to traditional stimulant therapy for Attention-Deficit/Hyperactivity Disorder (ADHD).

This presentation will impact the forensic community and/or humanity by educating forensic pathologists and toxicologists in the evaluation of atomoxetine levels in various postmortem fluids and tissues. This knowledge will be helpful, since atomoxetine is being used increasingly by prescribing physicians as a non-stimulant alternative to traditional drug treatment for Attention-Deficit/Hyperactivity Disorder

Atomoxetine (Strattera®, Lilly) is a selective norepinephrine reuptake inhibitor prescribed for the treatment of Attention-Deficit/Hyperactivity Disorder (ADHD) in children, adolescents and adults. It is the first non-stimulant drug-therapy option for ADHD. Three case reports are presented in which atomoxetine was detected in two individuals who died from causes unrelated to the drug and a third from the intentional ingestion of atomoxetine and other drugs. Postmortem blood levels of atomoxetine in the discussed cases ranged from 0.1 to 8.3 mg/L while the postmortem liver levels ranged from less than 0.44 to 29 mg/L.

Postmortem Fluid and Tissue Distribution of Atomoxetine

	Atomoxetine (mg/L or *mg/kg)						
	Aorta Blood	Femoral Blood	Vitreous	Bile	Urine	Liver*	Gastric*
Case 1	0.65	0.33	0.1	1.0	NA	3.9	0.54
Case 2	8.3	5.4	0.96	33	NA	29	860
Case 3	< 0.1	< 0.1†	NA	NA	< 0.1	< 0.44	NA

NA – not available; † - vena cava blood

Routine organic base drug screening detected presence of atomoxetine in the central blood of two cases presented. Screening analyses were performed on aliquots of blood using a liquid-liquid extraction. After the addition of alphaprodine (1 mg/L) as the internal standard, specimen aliquots were made basic by the addition of ammonium hydroxide (0.5 mL). Extraction was affected by the addition of *n*-butyl chloride:ether (4:1; 7 mL). The organic solvent was subjected to a back-extraction with 1 M sulfuric acid (2.5 mL). The aqueous phase was separated from the organic layer and was washed with hexane (2 mL). Removal of the hexane layer and further addition of ammonium hydroxide to the aqueous phase allowed the basic drugs to be drawn into *n*-butyl acetate (100 µL). The *n*-butyl acetate extracts were transferred to autosampler vials for analysis by gas chromatography (GC), equipped with a nitrogen-phosphorous detector (NPD), followed by definitive identification by full scan mass spectrometry (MS). Quantification of the original blood specimen and analysis of additional specimens collected in each case on the appropriate amount of specimen to fall with in the linear range of the assay followed the same extraction procedure with the addition of a standard curve and analyte-specific quality control, utilizing GC/NPD. Working methanolic spiking solutions of both standards (10, 100 µg/mL) and controls (10 µg/mL) were prepared by serial dilution from a 1 mg/mL stock solution prepared from the powder supplied from the pharmaceutical company. Calibration curves and quality control samples were created by spiking aliquots of drug-free blood with the working spiking solutions at the appropriate concentrations. Confirmation analyses utilized a 5-point calibration curve at concentration levels of 0.2, 0.5, 1.0, 2.0 and 4.0 mg/L, with a positive control sample at 0.5 mg/L. An aliquot of the drug-free blood was analyzed concurrently with each batch as a negative control. The assay was linear from 0.2 – 10 mg/L with a least squares linear regression analysis correlation coefficient (r^2) of 0.998 or better. The limits of quantitation and detection were 0.1 mg/L and 0.05 mg/L, respectively. Accuracy and precision studies conducted with a control spiked at 0.5 mg/L (3 x n=5) gave a mean within 8% of the target value (CV= 7%). GC/NPD was then performed; injections of 1 µL in the splitless mode were made with an inlet temperature of 275°C. Helium was the carrier gas at a flow rate of 6.7 mL/minute with an initial oven temperature of 120°C. This was followed by an increase of 15°C/min until 300°C, holding for 3 minutes. Drug confirmation by GC/MS was performed Split injections (3:1) of 1 µL were made with an inlet temperature of 275°C. Helium was the carrier gas at a flow rate of 1.4 mL/minute with an initial oven temperature of 70°C for 2 minutes. The oven temperature was then ramped at a rate of 15°C/minute until 300°C was reached where it was held for 7 minutes. Electron impact ionization was utilized in the scan mode, monitoring ions from 40-550 *m/z* ratio.

Atomoxetine is well absorbed after oral administration, with a bioavailabilty of 63% and is highly plasma protein bound (98% to albumin). Maximal plasma concentrations of atomoxetine occur 1-2 hours after dosing and its half-life is approximately 5 hours. Atomoxetine has a low volume of distribution (Vd) (0.85 L/kg) suggesting little tissue sequestration. Atomoxetine is metabolized primarily by cytochrome P450 (CYP) 2D6 to yield 4-hydroxyatomoxetine, which is subsequently glucuronidated. A second metabolite, *N*-desmethylatomoxetine is formed by the action of CYP2C19. 4-hydroxyatomoxetine has equipotent SNRI pharmacological activity to the parent drug but is only present in the plasma at very low concentrations. *N*-desmethylatomoxetine not only has less pharmacological activity than atomoxetine, but is also present in plasma at lower concentrations. The elimination half-life of the two metabolites is 6-8 hours. Greater than 80% of the dose of atomoxetine is excreted in the urine as 4-hydroxyatomoxetine-*O*-glucuronide with less than 17% of the dose appearing in the feces. Poor CYP2D6 metabolizers will display altered pharmacokinetic data.

Atomoxetine can be considered non-toxic at whole blood and liver concentrations below 1.3 mg/L and 5 mg/kg, respectively. Although the drug has a low volume of distribution, it appears to undergo postmortem redistribution with a mean central to peripheral ratio of 2.7 (range: 1.5 – 5.6).

Although this is a preliminary study, little is known through clinical trials or reports of the toxicity of atomoxetine in overdose; even less is known about postmortem toxicology. The presence of atomoxetine was considered an incidental finding in two of the cases presented while the third involved an intentional overdose. The corresponding postmortem fluid and tissue distribution of atomoxetine in addition to the significant anatomic findings autopsy are reported.

Atomoxetine, Postmortem, Forensic Science

K40 Postmortem Blood Concentrations Following the Oral Ingestion of Transdermal Fentanyl Patches (Duragesic®)

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The objective of this presentation is to describe the oral administration of transdermal fentanyl patches (Duragesic®) with resultant blood concentrations in seven deaths in the province of Ontario, Canada.

This presentation will impact the forensic community and/or humanity by alerting the forensic community to an unusual opioid abuse practice: the ingestion of fentanyl patches. Detailed case reports of seven deaths will assist forensic toxicologists in the interpretation of postmortem blood fentanyl concentrations that may arise following this route of administration.

Introduction: Fentanyl is a synthetic narcotic analgesic that is available in the form of a transdermal patch for the management of chronic pain. Transdermal patches contain 2.5-10 mg fentanyl and provide a dose of 25-100 µg/hr for 72 hours. Therapeutic serum concentrations following transdermal application have been reported up to 5 ng/mL. Since the introduction of the transdermal system to treat chronic pain, the patches are increasingly being found in the opioid-abusing population. There have been numerous reports of abuse of the transdermal delivery systems through the application of multiple patches or by intravenous injection of the patch contents. In addition, there are a limited number of reports where abuse of these transdermal patches was achieved via ingestion or inhalation.

Methods: Fentanyl-related deaths following the oral ingestion of transdermal patches were retrospectively identified from the files of the Toxicology Section of the Centre of Forensic Sciences, which provides the sole toxicology testing for coroner's investigations in the province of Ontario (approx. population 12 million). Inclusion criteria were: time period between 2002 and 2004 and the detection of fentanyl in postmortem blood. Further information pertaining to the circumstances of death, autopsy findings, and cause and manner of death was obtained from the Office of the Chief Coroner of Ontario. The route of fentanyl administration was classified as oral based on both observations of individuals chewing fentanyl patches prior to death or the finding of fentanyl patches in the oral cavity or pharynx during autopsy. Fentanyl was extracted from blood samples by liquid/liquid extraction and quantitation was performed using gas chromatography-mass spectrometry in the electron ionization mode.

Results & Discussion: A total of 119 fentanyl-related deaths were identified for the period of 2002 to 2004, of which there were seven cases where the route of administration was classified as oral. The seven decedents comprised three females and four males with ages ranging from 32 to 51 years. Postmortem blood fentanyl concentrations were determined in all cases and ranged from 7 to 97 ng/mL with a mean blood concentration of 28 ng/mL. There were two cases in which death was attributed solely to fentanyl overdose. The first was a 42-year-old male found dead in bed with numerous small pieces of a transdermal patch in the oral cavity and a heart blood fentanyl concentration of 22 ng/mL. The second case was that of a 20-year-old woman who had shared and ingested the contents of a 10 mg fentanyl patch the night before her death. Postmortem fentanyl was measured in a femoral blood sample at a concentration of 13 ng/mL. The blood fentanyl concentrations in these two cases are higher than those observed following therapeutic administration of transdermal patches and are comparable to concentrations in cases of fentanyl overdose following excessive transdermal application.

In three other cases, fentanyl was present in combination with ethanol and death was considered to have been the result of combined ethanol/fentanyl toxicity. The concentrations of fentanyl were 7, 8 and 28 ng/mL with corresponding blood alcohol concentrations of 209, 171 and 160 mg/100mL, respectively. The final two cases that will be presented comprise a case of mixed drug toxicity in which the fentanyl concentration was 97 ng/mL and a case in which administration was via both the transdermal and oral route of administration. In the latter case, a fentanyl concentration of 19 ng/mL was detected and the medical cause of death was due to a combination of fentanyl toxicity and a perforated duodenal ulcer.

Although oral ingestion accounted for less than 6% of the total number of fentanyl-related deaths in Ontario between 2002 and 2004, the seven deaths associated with this practice illustrate that toxic blood concentrations can occur following this route of administration. Detailed case reports of these cases will expand on this unusual means of Duragesic® abuse and illustrate the range of blood fentanyl concentrations that may be expected.

Fentanyl, Postmortem, Oral Abuse

K41 Toxicology of Deaths Associated With "Ecstasy"

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After attending this presentation, attendees will understand the interpretation of MDMA concentrations in postmortem toxicology.

This presentation will impact on the forensic community and/or humanity by demonstrating how MDMA concentrations in postmortem toxicology can only be interpreted in the context of other information. Considerable overlap exists between fatal concentrations and those seen in deaths from trauma.

3,4-Methylenedioxymethylamphetamine (MDMA), more popularly known as "Ecstasy" has been a widely used recreational drug in the UK since the beginning of the 1990s [1]. Deaths were first reported in the UK in the early 1990s at Raves and clubs, with deaths attributable to hyperpyrexia, water intoxication and cardiac dysrhythmias. Deaths from hepatic necrosis have also occurred. This study of deaths seen in Yorkshire in the North of England reports the toxicological findings where MDMA and related drugs have been found on post-mortem toxicology.

17 deaths were attributed to the effects of MDMA or MDEA (3,4-methylenedioxyethylamphetamine) alone. In 13 deaths collapse was rapid. Peripheral blood (femoral) analysis in these rapid deaths revealed MDMA concentrations of 0.478 mg/L – 53.9 mg/L. The mean concentration was 8.43 mg/L, median 3.49 mg/L. Two cases were also positive for MDEA with concentrations of 3.4 mg/l and 3.5 mg/L. 3,4-Methylenedioxyamphetamine (MDA) concentrations ranged from 0.012 – 8.5 mg/L (mean 1.5 mg/L, median 0.79 mg/L). Other drugs found were cannabinoids (6), amphetamine (5), ethanol (5), cocaine (1), LSD (1), benzodiazepine (1).

In 29 cases, death was attributed to polydrug use, MDMA (27), MDEA (1), MBDB (1). In 22 cases MDMA was recorded in blood, range

0.04 to 41.5 mg/L. The mean value in these deaths was 2.90 mg/L, median 0.76 mg/L. The other principal drugs in these cases were cannabinoids (16), ethanol (12), heroin (11), benzodiazepines (9), amphetamine (8), antidepressants (6), methadone (5), cocaine (5), GHB (2).

In 29 cases death was traumatic, homicide (8), vehicular collision (10), fall from height (6), drowning (4), hypothermia (1). In 24 cases MDMA was found in blood with concentrations ranging from 0.035 mg/L to 4.81 mg/L, mean 0.862 mg/L, median 0.483 mg/L. Other drugs found were ethanol (17), cannabinoids (10), amphetamine (5), cocaine (4), antidepressants (2), ketamine (1).

In conclusion, MDMA showed a wide range of concentrations. Higher concentrations were seen where death was attributed to the effects of MDMA alone, but considerable overlap exists between concentrations seen in drug related deaths and deaths due to trauma.

References:
1. Milroy CM. Ten Years of "Ecstasy." Journal of the Royal Society of Medicine. 1999; 92: 68-72.

Ecstasy, Death, Toxicology

K42 Suicide by Inhalation of Freons: Detection of in a Partially Decomposed Body

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The goals of this presentation are to present an unusual mechanism of suicide by freons inhalation and present a method for detection of freons in body fluids and tissues.

This presentation will impact the forensic community and/or humanity by demonstrating an unusual method of suicide and demonstrates that detection of volatile freons can still be achieved in a partially decomposed body.

This case describes a fatality due to suicidal inhalation of two widely available refrigerant gases: chlorodifluoromethane (HCFC-22) and 1,1,1,2-tetrafluoroethane (HFC-134a), a combination that has not been previously reported in forensic literature. A 51-year-old white man was found dead at home in the bathroom on the floor in a large plastic trash bag that covered the entire body. Two commercial 30-pound gas tanks containing the gases were suspended with two separate plastic tubes connected to the valves of the tanks that were running into the bag. The valves on both tanks were fully opened. The deceased was in an early generalized state of decomposition. There are no reports in forensic literature describing detection of refrigerants in a partially decomposed human body. Volatile gas analysis was performed using gas chromatography mass spectrometry. The chromatographic column used was a HP-5 MS capillary column (cross-linked 5% phenyl-methylsilicone, 30 meter length, 0.25 mm internal diameter, 0.25 um film thickness). Aliquots of the biological samples (1 gram of tissue, 1.0 ml of blood and bile) were sealed in 2 ml glass vials and heated at 30°C for 30 minutes; 50 uL of the headspace gas was aspirated from each vial and injected into the GC/MS system. The retention times of HCFC-22 and HFC-134a were nearly identical, 1.76 and 1.78 minutes, respectively. A full scan of commercially purchased standards identified the ions unique to the compounds. A full scan of the peak showed all major ions of both compounds were identified in the following specimens: blood, bile, liver, spleen, heart muscle, thigh muscle, subcutaneous fat, brain, kidney, lung, pancreas, spinal cord, and thyroid. The relative amount of each gas in the samples was determined by comparison to the peak area obtained from injection of precise volumes of the 98% pure gas using the following ions in SIM mode (HCFC-22, 67, 85, 47, and HFC-134a 83, 63). The ratio of HCFC-22 and HFC-134a ranged from 1.4 in muscle to 4.0 in liver.

Freons, GC/MS, Suicide

K43 Absence of Elevated Carboxyhemoglobin Following Inhalation of Automobile Exhaust

Rebecca A. Jufer, PhD, Barry S. Levine, PhD, Deborah Green Johnson, BS, Mary Ripple, MD, and David R. Fowler, MD, State of Maryland, Office of the Chief Medical Examiner, 111 Penn Street, Baltimore, MD 21201*

After attending this presentation, attendees will become familiar with cases of asphyxia due to inhalation of automobile exhaust without an elevated carboxyhemoglobin concentration.

This presentation will impact the forensic community and/or humanity by providing information on cases of inhalation of automobile exhaust with atypical findings.

The objective of this presentation is to present three cases of suicide by inhalation of automobile exhaust that did not result in elevated carboxyhemoglobin levels.

Carbon monoxide is a colorless and odorless gas that is produced as a result of incomplete combustion of organic materials. It is a common component of automobile exhaust. Other components of automobile exhaust include nitrogen, carbon dioxide, water vapor, hydrocarbons, and nitrogen oxides. U.S. air pollution control programs that were initiated in the 1970s resulted in engine design changes and emission control devices that were designed to reduce carbon monoxide, volatile organic compounds, and nitrogen oxides, the more harmful components of automobile emissions. Additionally, changes in the formulation of gasoline have contributed to the reduction of these components of automobile exhaust. These changes may alter the typical findings in some cases of automobile exhaust inhalation, as illustrated by three cases received at the OCME in Baltimore, MD.

Cases 1 & 2: A husband and wife (aged 57 and 55 years, respectively) were found deceased in a running 2001 Chevy Tahoe that was parked in the backyard of a residence. A flexible dryer vent hose was attached to the car exhaust and inserted into the right passenger window, with towels placed into the open areas around the hose. The decedents were seated in the rear seat of the vehicle with two deceased Yorkie dogs between them. There were no signs of a struggle and neither victim showed signs of trauma. The position of the bodies was consistent with the victims dying while seated in the vehicle. Two suicide notes were recovered at the scene, one in the vehicle and one inside the residence. Blood was collected from the decedents and sent to the laboratory for analysis.

Case 3: A 52-year-old male was found unresponsive in a running 1983 Ford Ranger. A garden hose extended from the rear exhaust into a rust hole in the passenger floorboard. The hose was attached to the exhaust with tinfoil and medical tape. Two notes describing his intentions were located, one in the house and one on the seat of the truck. The subject had a history of heroin abuse and had discussed his desire to commit suicide with his father approximately six months earlier. He was resuscitated and transported to the hospital where he died one hour later. Blood was collected and submitted to the laboratory for analysis.

Blood specimens were analyzed for carboxyhemoglobin by gas chromatography. Two aliquots were prepared for each specimen. The first aliquot was sealed in a headspace vial. The remaining aliquot was saturated with carbon monoxide using a tonometer and then transferred to a headspace vial. Potassium ferricyanide was added to each sample to separate carbon monoxide from hemoglobin. A sample of the vial headspace was injected onto a 5A molecular sieve column, reduced to methane with a

nickel catalyst and detected with a flame ionization detector. Matrix blank and quantitative controls were included in each batch. Percent carbon monoxide saturation was calculated by comparing the response of the unsaturated sample to the saturated sample.

In cases 1 & 2, toxicological analysis indicated a blood carbon monoxide saturation of 3% for the female decedent and 4% for the male decedent. Other toxicological findings from a comprehensive drug screen and volatiles screen were unremarkable. The toxicological analysis did not include a cyanide screen. The medical examiner ruled that the cause of death was asphyxia due to inhalation of car exhaust and the manner of death was suicide in both cases.

In case 3, carbon monoxide saturation was less than 1%. In addition, ethanol and other volatiles were not detected at a cutoff of 0.01 % (w/v) and free morphine was not detected at a cutoff of 25 µg/L. The toxicological analysis did not include a cyanide screen. The medical examiner ruled that the cause of death was asphyxia due to inhalation of car exhaust and the manner of death was suicide.

The cases presented indicate that suspected carbon monoxide poisoning cases may present with atypical findings. In such cases, a comprehensive toxicological screen and scene investigation should be conducted whenever possible to rule out other causes of death.

Automobile Exhaust, Carbon Monoxide, Asphyxia

K44 Toxicological Testing of Emergency Responders Following the World Trade Center Attack

Michael F. Rieders, PhD, Robert A. Middleberg, PhD, Eric F. Rieders, PhD, and Lee M. Blum, PhD, National Medical Services, Inc., 3701 Welsh Road, Willow Grove, PA 19090*

After attending this presentation, attendees will learn of the specimen collection and analysis methods needed to quantify beryllium, mercury, and polychlorinated biphenyls (PCBs) in exposed workers; understand the toxicology of these substances, and their sources along with other toxic substances that were released from the World Trade Center collapse.

This presentation will impact the forensic community and/or humanity by providing results of toxicological testing of workers following exposure incidents such as this and may be useful in assessing the adequacy of the respiratory protection equipment to help improve safety equipment in the future. Results may also be used to predict the risk of subsequent potential health effects and to implement prevention programs and to reduce morbidity and mortality from exposure. Human biological monitoring results may also be useful in re-engineering building materials and reducing production of toxic aerosols in the event of fire or collapse.

Post-incident testing of workers for exposure to toxic substances is essential in order to determine whether or not such substances caused or contributed to morbidity or mortality of individual workers. Blood and urine specimens must be collected in an appropriate manner to avoid contamination and degradation. In an emergency action such as the second attack on the World Trade Center leading to collapse of the buildings, pre-shift specimens from emergency responders were not collected. Such specimens are important in determining workers body burden prior to the exposure event. Post-event specimens were collected for testing of specific analytes.

Specimen collection included 5,314 urine specimens that were tested for beryllium and mercury; and 5,312 serum specimens were tested for PCB's. Tests for specific gravity and creatinine were performed on urine samples to validate the samples and for calculating final concentrations of the metals using a creatinine correction algorithm. Beryllium and mercury were analyzed by inductively coupled plasma/mass spectrometry (ICPMS) and polychlorinated biphenyls were analyzed by gas chromatography using electron capture detection; calculation was based on Arochlor 1260. All three analytes may be found normally at low concentrations in the general population. Beryllium is elevated in tobacco smokers whose levels may be double the concentration of non-smokers. Smoking history and other factors need to be recounted for the final interpretation of test results.

A statistical analysis of test groups showed beryllium, mercury and PCB concentrations to be within that expected for the general population for almost all specimens tested. Only a few of the 5000 or so specimens tested demonstrated results that appeared elevated from reference ranges, yet not within the realm of where toxicity is expected.

Results of toxicological testing of workers following exposure incidents such as this may be useful in assessing the adequacy of the respiratory protection equipment to help improve safety equipment in the future. Results may also be used to predict the risk of subsequent potential health effects and to implement prevention programs and to reduce morbidity and mortality from exposure. Human biological monitoring results may also be useful in re-engineering building materials and reducing production of toxic aerosols in the event of fire or collapse.

Heavy Metals, PCB's, World Trade Center

K45 6-Acetylmorphine in Hair: Self-Reported Heroin Users Not Considered Positive Using Proposed Federal Guidelines

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After attending this presentation, attendees will learn the metabolic profile of heroin in hair specimens and understand the proposed Federal Guidelines for testing opiates in hair.

Guidelines and proposals are sometimes implemented without adequate regard for the relevant science. This presentation will impact the forensic community and/or humanity by showing that a significant number of admitted heroin users would not be considered positive under workplace testing rules if the proposed requirement for the presence of morphine is implemented.

Methods: Heroin abuse is associated with adverse health conditions, including fatal overdose, collapsed veins, and, when injected, infectious diseases, including HIV/AIDS and hepatitis. The central nervous system is depressed following heroin intake, and mental functions are generally impaired. Long-term effects include heart infections, abscesses and liver disease. Pulmonary complications may result from the poor health condition of the abuser, as well as from heroin's depressing effect on respiration.

This study was designed to determine whether the proposed Federal Rules for one of the "alternative" matrices, hair, would effectively identify heroin users. The proposed Federal guidelines for morphine, codeine and 6-acetylmorphine (6-AM) state that a hair specimen containing at least 200 pg/mg of 6-AM cannot be reported as positive unless it also contains at least 200 pg/mg of morphine.

The study enrolled 203 subjects, approximately half of whom admitted to opiate use, half who did not. Each subject provided a hair specimen taken from the head at the time of interview. Information on drug use, including time of last use, frequency of use, ethnicity, age, sex and hair color were recorded for each subject. The specimens were analyzed for morphine, codeine and 6-acetylmorphine using immunoassay and gas chromatography-mass spectrometry. While the analysis of various opiates in hair has been previously published, this is the first study where the positivity rate was determined according to proposed Federal guidelines.

Results: Mono-acetylmorphine (6-AM) was the major metabolite detected in hair following heroin use, and in all except three samples it was present in higher concentrations than morphine. Only one sample from an admitted heroin user did not contain 6-AM, and there were no samples containing only morphine. Overall, the mean morphine concentration detected in hair was 780 pg/mg; (median 407 pg/mg). The mean codeine level was1174 pg/mg; (median 481 pg/mg) and the mean concentration of 6-AM in the hair samples was 1904 pg/mg; (median 828 pg/mg). Morphine was present in 34 of the 52 positive samples from the self-reported group (65.3%); codeine in 46/52 (88.4%) and 6-AM in 38/52 (73%).

In the self-reported opiate using population, 45 hair samples confirmed positively for opiates under the proposed rules. However the other seven (15.5%) contained 6-AM at concentrations higher than 200 pg/mg, so would not have been considered positive under the regulations.

In the self-reported non-drug using population, 7 specimens (7%) were positive under the guidelines. Five of these contained codeine at levels higher than 200 pg/mg and the other 2 contained codeine, morphine and 6-AM.

Currently, the proposed Federal guidelines require morphine to be present as well as 6-AM in hair specimens in order to be reported as positive. The data shows that this will cause approximately 15% of heroin users to go undetected. Based on this study, and supported by the literature, morphine is not the predominant metabolite detected in hair following heroin use. Therefore, if the detection of heroin users is the focus of the Federal program, the presence of 6-AM alone in hair should be considered a positive result.

Summary: When specimens were analyzed according to the levels proposed in the Federal guidelines for alternative samples, hair failed to identify seven self-reported heroin users. Even though 103 subjects admitted opiate intake, not all were heroin users, some admitting to hydrocodone or oxycodone intake. However, of the seven who admitted frequent heroin use, all hair specimens contained measurable amounts of 6-AM, confirming their admission. Under the Federal Guidelines, these individuals would not have been reported as positive. It has been suggested that hair is a superior matrix to urine for the detection of drug users, however for this to be true, appropriate detection levels must be mandated, and the stand-alone presence of 6-AM must be considered a positive result.

6-Acetylmorphine, Federal Guidelines, Hair Analysis

K46 The Detection of 11-nor-Δ9-THC-9-Carboxylic Acid (THC-COOH) in Hair and Urine

Christine Moore, PhD, Sumandeep Rana, MS, Cynthia Coulter, BS, Michael Vincent, MS, and James Soares, PhD, Immunalysis Corporation, 829 Towne Center Drive, Pomona, CA 91767*

After attending this presentation, attendees will understand how to analyze 11-nor-Δ^9-THC-9-carboxylic acid (THC-COOH) in hair using a two dimensional gas chromatographic system coupled to a single quadropole mass selective detector

The detection of marijuana in hair at meaningful concentrations has currently been limited to analysis using triple quadrupole mass analyzers. This presentation will impact the forensic community and/or humanity by describing the application of two dimensional gas chromatography to a toxicological problem, allowing the analysis of drugs and metabolites at extremely low levels. The modifications can be applied for many different applications in toxicology

Methods: Tetrahydrocannabinol (THC) is the active ingredient in marijuana and is generally administered orally or by smoking, resulting in euphoria and hallucinations. Since its main metabolite, 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), is acidic, its incorporation into the hair shaft is not as extensive as that of more basic drugs such as cocaine or methamphetamine. Hence, the detection of marijuana metabolite, THC-COOH, in hair is extremely difficult, due to the very low levels incorporated and the sensitivity of detection. Even though the use of two-dimensional chromatography has been applied for many years in the oil and petroleum industry, its application to forensic toxicological problems was first described in 2003, and coupling to mass spectrometry for the detection of drugs of abuse was reported for the first time in 2004. The approach to the problem of inadequate detection levels using a single stage quadrupole mass spectrometer was to make sufficient small improvements over the entire assay, so that the final required detection limit could be routinely achieved.

Gas Chromatography - Two dimensional GC: The application of a prior separating column to the assay allowed the background associated with the hair extract to be spread out over a longer time frame. Once the analyte retention time on the first column had been determined, the pressure switch (Dean's switch) was turned on to divert the flow, and turned off 0.4 minutes later. This created a narrow "window" of the effluent from the first column containing the analyte to be passed to the analytical column with minimal background. The second analytical column was of a different polarity than the first and provided a further separation of the analyte from potential interferences.

Cryogenic Focusing: The fraction from the first column was selectively transferred to the analytical column where a cryogenic trap focused the peak of interest. The column was cooled as the analyte entered, effectively "cold-trapping" the drug. The focuser was then heated quickly allowing the peak of interest to advance through the analytical column and enter the mass spectrometer. This resulted in a much sharper chromatographic peak, producing an improved signal to noise ratio.

Mass Spectrometry: Chemical ionization provided a more specific and selective ionization of analytes than electron impact ionization, by enhancing the signal and lowering noise generated by potential interferences. The greatest potential gains were found in applying electron capture chemical ionization (ECCI) using ammonia as the reagent gas. The low gas pressure provided sufficient fragmentation to allow the monitoring of two ions for the drug and internal standard. The modifications described were necessary to in order to analyze samples at the proposed Federal guideline cut-off of 0.05 pg/mg.

Our study enrolled 156 subjects, all of whom admitted to recent marijuana use. Each subject provided a urine sample and a hair specimen taken from the head at the time of interview. Information on drug use, including time of last use, frequency of use, ethnicity, age, sex and hair color were recorded for each subject. Hair samples were analyzed at Immunalysis Corporation; urine samples were analyzed by a reference laboratory.

Results: Of the 156 specimens collected, 46 (29%) of the samples were positive using hair, urine or both. Eight (5.1%) were positive using urine only, nine (5.7%) were positive via hair only, twenty-eight (17.9%) were positive in both matrices, and the remaining 71% were negative. One sample (# 50) had no data for the urinalysis.

The frequency of use reported by the subjects ranged from as high as 10 times per day (subjects 50 and 151) to as infrequently as 3 times per week (subjects 19, 58, 134, 152 and 154). Overall, there appeared to be very little correlation between the self-reported use of marijuana and the concentrations detected in hair or urine.

Summary: The detection of THC-COOH in hair can be achieved at a similar positivity rate to urine when a low enough detection limit is used. Using a modified GC/MS system, THC-COOH was identified in hair at the level of 0.05 pg/mg, as mandated in the proposed Federal guidelines.

Sample ID	Hair result (pg/mg)	Urine result (ng/mL)
1	**Negative**	129
2	0.42	352
4	0.41	427
10	0.29	**Negative**
17	1.22	598
18	2.51	405
19	**Negative**	135
24	0.12	**Negative**
25	0.18	262
26	0.45	181
29	0.16	**Negative**
30	0.95	522
35	0.13	**Negative**
37	0.88	634
39	0.1	**Negative**
49	0.18	312
50	0.89	Data unavailable
51	0.62	407
57	0.24	489
58	**Negative**	297
65	**Negative**	307
69	0.68	613
72	0.49	505
79	0.45	**Negative**
81	0.12	**Negative**
96	**Negative**	465
97	1.12	592
99	0.11	**Negative**
100	0.52	397
103	1.04	686
109	0.15	500
110	0.44	299
114	**Negative**	136
122	0.99	530
125	**Negative**	463
126	**Negative**	200
129	0.56	810
130	0.59	772
131	0.3	472
134	0.2	**Negative**
138	1.08	621
139	0.23	70
146	0.35	277
151	0.95	586
152	0.22	149
154	1.16	234

THCA, Two-Dimensional GC, Hair Analysis

K47 Buprenorphine and Norbuprenorphine Concentrations in the Hair of Opiate-Dependent Pregnant Women and Their Newborn Infants

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After attending this presentation, attendees will have a better understanding of the application of hair analysis for the determination of buprenorphine and norbuprenorphine in women maintained on buprenorphine and their infants exposed in utero.

This presentation will impact the forensic community and/or humanity by addressing the usefulness of hair analysis as a tool for the determination of illicit and therapeutic drugs.

Accurate identification of in utero illicit and therapeutic drug exposure has important implications to mothers and infants. Buprenorphine, a partial μ agonist, is under investigation as a pharmacotherapy for treating opioid dependence in pregnant women. Hair testing may be a useful tool for the determination of drug exposure during pregnancy; however, data are limited on the disposition of buprenorphine and norbuprenorphine in maternal and infant hair.

This study examined buprenorphine and norbuprenorphine concentrations in hair obtained from nine (8 African-American, 1 Caucasian) buprenorphine-maintained pregnant women and four of their infants. Women received 4 – 24 mg daily sublingual buprenorphine throughout gestation, an average of 16.3 ± 2.8 weeks; 13 – 21 (mean ± SD; range). Mean total maternal buprenorphine dose was 1742.4 ± 385.3; (range, 1204 – 2270 mg). Mean cumulative third trimester maternal buprenorphine dose was 1347.6 ± 241.6 mg; (range, 920 – 1672mg).

Maternal hair specimens (N=52) were collected, root to tip, approximately every 4 weeks throughout enrollment and stored at -20°C until time of analysis. Specimens were analyzed at the Center for Human Toxicology, Salt Lake City, UT utilizing liquid chromatography-tandem mass spectrometry with limits of quantification of 3.0 pg/mg for buprenorphine and norbuprenorphine in hair. Prior to analysis, hair specimens were cut into 3 cm segments, representing approximately 3 months of hair growth. Hair specimens were washed twice with 5 mL of methylene chloride at ambient temperature. Of 52 washed maternal hair specimens, 40 were positive for buprenorphine (46.4 ± 33.74; 8.6 – 161.8 pg/mg) and 41 positive for norbuprenorphine (607.5 ± 496.9; 8.2 – 1733.7 pg/mg) in the first segment of hair (closest to the scalp). Ratios of buprenorphine to norbuprenorphine ranged from 0.04 to 0.70 (0.14 ± 0.17). When sufficient amounts of hair were available (N=20), specimens also were analyzed without washing. Of the 20 unwashed specimens, 18 were positive for buprenorphine (47.7 ± 35.6; 4.6 – 151.3 pg/mg) and 17 for norbuprenorphine (714.0 ± 554.3; 46.5 – 2018.6 pg/mg) in the unwashed first hair segment. There was no statistically significant difference between the concentrations of buprenorphine (p=0.64) or norbuprenorphine (p= .86) in the washed and unwashed hair specimens. Ratios of buprenorphine to norbuprenorphine in the unwashed specimens ranged from 0.04 – 0.49 (0.15 ± 0.14) and were not significantly different in washed hair specimens (p=0.89).

Infant hair specimens (N=4) were collected within 48 hours of delivery and were not washed prior to analysis. Neonatal hair was not washed because drug concentrations could have been lower than in the mother, and it was not known if washing would remove excess amounts of drug due to the fine texture of the hair, and there was an insufficient amount

of hair to test both washed and unwashed specimens. All infant specimens were positive for buprenorphine (54.7 ± 21.7; 36.8 – 82.1 pg/mg) and norbuprenorphine (785.6 ± 190.2; 579.9 – 1037.1 pg/mg). The ratio of buprenorphine to norbuprenorphine in infant hair ranged from 0.05 to 0.08 (0.07 ± 0.02). There was no correlation between maternal total buprenorphine dose and buprenorphine (r^2=0.05, p=0.95) or norbuprenorphine (r^2=0.18, p=0.82) concentrations in infant hair. There also was no correlation between 3rd trimester maternal buprenorphine dose and buprenorphine (r^2=0.29, p=0.71) or norbuprenorphine (r^2=0.09, p=0.91) in infant hair.

Higher concentrations of norbuprenorphine as compared to buprenorphine were found in maternal and neonatal hair following daily maintenance doses of buprenorphine to opiate-dependent pregnant women. Washing maternal hair with methylene chloride did not significantly decrease parent or metabolite concentrations in the specimens. Although buprenorphine pharmacotherapy offered the opportunity to evaluate dose-concentration relationships in this vulnerable population, no significant correlations were observed between maternal buprenorphine dose and buprenorphine or norbuprenorphine concentrations in maternal or neonatal hair. Research supported by NIDA R01-12220 and NIH DA 09096.

Buprenorphine, Hair, In Utero

K48 Three Gamma Hydroxybutyrate-Related Deaths

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The goal of the presentation is to identify and describe the characteristics of three, HCMEO Gamma Hydroxybutyrate (GHB) - related deaths occurring within the period of 2000- 2005.

This presentation will impact the forensic community and/or humanity by demonstrating how the interpretation of postmortem GHB can be a challenge to determine its significance in the cause and manner of death.

Gamma-Hydroxybutyric acid had been used clinically, beginning in the early 1960s, as an anesthetic and hypnotic agent. It is now classified as a Schedule I drug in the United States and has no currently approved medical use. This reclassification was made in 2000 as a result of its abuse as an alternative to anabolic steroids by body builders, and by others, for its central nervous system depressant effects: drowsiness, dizziness, visual disturbances, amnesia and loss of consciousness. GHB has become a popular drug of abuse in association with sexual assaults. Researchers have reviewed twenty-three Harris County Medical Examiner's Office (HCMEO) cases, from the five-year period between 2000 and 2005, in which GHB was detected. GHB was determined to be the cause of death in three of these cases.

Interpretation of postmortem GHB is a formidable challenge. GHB occurs endogenously in most mammalian tissues, as a product of postmortem degradation, or as a metabolic product of the inhibitory neurotransmitter, gamma-aminobutyric acid. It is generally not detected at significant concentrations in blood or urine from living persons and postmortem production can be reduced through use of preservatives. Peak plasma concentrations can occur within 20-40 minutes and peak urine concentrations occur within 4 hours of drug use. The half-life in blood is less than one hour and its duration of action are three to four hours. Less than 5% of a dose is eliminated unchanged in urine and it is generally undetected by twelve hours after administration. GHB may also be administered in the form of Gamma-Butyrolactone, (GBL) and this substance may be observed in urine, as evidence of the dosage form or as a product of spontaneous cyclization.

The three GHB-related cases from the Harris County Medical Examiner's Office are described in Table 1.

Table 1: Three Harris County Medical Examiner GHB Cases

Case Facts: **Age/Race/Gender**	Case 1 17 yrs. White Male	Case 2 25 yrs. White Male	Case 3 34 yrs. White Male
History of Illegal Drug Use	No	Cocaine & Ecstasy	Cocaine & GHB
Toxicology Results: **Blood GHB**	108 mg/L	328 mg/L	701 mg/L
Urine GHB	80 mg/L	5798 mg/L	761 mg/L
Urine GBL	<10 mg/L	50 mg/L	28 mg/L

The circumstances surrounding these three deaths were similar. In each case, the decedents had been partying with friends. Two of the decedents were later found dead at their residences and one was found at a friend's house. The time elapsed from last seen alive ranged from 5 to 12 hours. Case number 1, a seventeen-year-old male, had a history of depression and attention deficit disorder. Fluoxetine and diazepam were detected in the blood of this individual and were included in the cause of death. Alcohol or other drugs were not detected in any of these cases.

External or internal signs of trauma were not observed at autopsy. Congestion of organs was noted in all three cases and two had evidence of pulmonary edema.

GHB was detected and quantified in blood and urine by gas chromatography / mass spectrometry of liquid-liquid extracts after acid catalyzed cyclization to GBL. GBL, in urine, was determined by GC/MS, by extraction prior to acid treatment.

The cause of death in Case 1 was assigned as combined toxic effects of GHB, benzodiazepines, and fluoxetine. In cases 2 and 3, the cause of death was acute GHB toxicity. In all three cases the manner of death was accident.

Gamma-Hydroxybutyrate (GHB), Gamma-Butyrolactone, Cause & Manner of Death

K49 Prevalence of Carisoprodol, Methadone, Oxycodone and Zolpidem in Subjects Suspected of Driving Under the Influence of Drugs (DUID) by Enzyme Linked Immunosorbent Assay

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After attending this presentation, attendees will be aware of the prevalence of carisoprodol, methadone, oxycodone and zolpidem in the blood specimens from drivers in the state of Arizona.

This presentation will impact the forensic community and/or the public by demonstrating how the toxicological analysis for carisoprodol, methadone, oxycodone and zolpidem will significantly improve the safety of the population, particularly road-users in the state of Arizona.

Methods: Driving under the influence of drugs and/or alcohol is a major problem in public safety. Enzyme linked immunosorbent assays (ELISA) are currently used to screen for the presence of barbiturates, benzodiazepines, opiates, cocaine, methamphetamine and THC in whole blood. This study was designed to determine whether the prevalence of carisoprodol, methadone, oxycodone and zolpidem warranted their inclusion in the initial immunoassay screen performed in the subject population. The study evaluated 1109 consecutive cases submitted over a 5 month time period. Cut-offs were established which appeared to reflect a potentially impairing concentration, as well as a concentration which could be confirmed using gas chromatography-mass spectrometry (GC/MS). The established cut-off levels were carisoprodol 1000 ng/mL; methadone 100 ng/mL; oxycodone 25 ng/mL and zolpidem 25 ng/mL. The screening

prevalence was compared with the documentation supplied by the drug recognition experts or arresting officer, when available.

Results: Of the 1109 cases evaluated, 55 (4.9%) contained carisoprodol; 16 (1.4%) methadone; 51 (4.6%) oxycodone; and 9 of 946 cases (1%) were positive for zolpidem. Zolpidem was added to the panel at a later date; hence the number of specimens tested is lower. Not surprisingly, the most prevalent drug detected was marijuana, which was found in 47% of the cases. Methamphetamine was found in 30%. Benzoylecgonine and benzodiazepines each were detected in approximately 13% of the samples. The current opiate assay, the Immunalysis Opiates Direct ELISA Kit, is approximately 21% cross-reactive to oxycodone and in this study had 89 (8%) cases. The lowest class prevalence of drugs found was barbiturates at 2%. The GC/MS confirmation rates for these prevalence study assays are as follows:

Assay	Confirmation	Analytes found
Zolpidem	89 %	Zolpidem
Carisoprodol	96 %	Carisoprodol & meprobamate
	2 %	Meprobamate only
Methadone	100 %	Methadone
Oxycodone	25 %	Oxycodone
	18 %	Hydrocodone
	12 %	Codeine
	8 %	Morphine
	2 %	Codeine & morphine

15 (29%) of the presumptive positive oxycodone cases were not confirmed by the current GC/MS procedure and one case each for both carisoprodol and zolpidem. In many cases, multiple drugs were detected.

Summary: The additional ELISA screening has proven to be an effective approach to identify specimens for confirmation of prescription medications that have demonstrated impairing effects in the driving population. It has given this laboratory a preliminary screening test that is less labor intensive than and complementary to GC/MS. These results demonstrated that carisoprodol has a higher occurrence than the barbiturate class in the current screening set-up. The prevalence of methadone and zolpidem are not as high as expected. Zolpidem may be expected to rise based on the interest growing for this generation of drug type. Methadone has not increased as much as expected based on trends in other regions of the country. Oxycodone was not as prevalent as other parts of the country. It has not been decided to implement the use of the oxycodone assay because the majority of the positives were also identified by the current opiate assay. The differences between the two screening assays could be explained by the higher cutoff currently used for the opiate assay of 50 ng/mL. Several of the oxycodone positive samples were positive for opiates other than oxycodone.

Carisoprodol, Methadone, Zolpidem

K50 Detection and Quantification of Low Levels of Benzoylecgonine in Equine Urine

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After attending this presentation, attendees will understand some principles of testing race horses for cocaine and other drugs.

This presentation will impact the forensic community and/or humanity by providing understanding of a problem of so-called low level cocaine concentrations in horse urine.

Cocaine (COC) is a local anesthetic and psychostimulant plant alkaloid widely abused by humans by IV injections, snorting or smoking. After administration, COC is quickly and completely metabolized and excreted in urine. Benzoylecgonine (BE) is a primary COC metabolite detected in human and equine urine. COC has no accepted therapeutic applications in equine veterinary practice. Many horse racing toxicology laboratories in the United States and in other countries occasionally detect low concentrations of BE (<150 ng/mL) in urine samples collected from winning animals. It is known that these very low BE concentrations in horse urine are a result of an accidental transfer of COC from humans or environmental contamination rather than premeditated administration to increase a horse's performance during the race. In response to the controversy of very low BE concentrations in urine, in February 2005 the Illinois Racing Board issued new rules establishing the threshold level of 150 ng/mL for BE in urine. According to the new rule, the first three positive BE laboratory reports below 150 ng/mL are accompanied by increasing fines ($250, $500, and $1000, respectively). The presence of BE in urine at a concentration equal to or higher than the threshold level is treated as a Class 1 drug as defined in the Association of Racing Commissioners International Uniform Classification Guidelines for Foreign Substances.

Methods: A solid phase extraction method for extraction of BE from 2 mL of equine urine followed by EI-GC-MS analysis after derivatization with BSTFA with 1% TMCS was developed and validated. D_3-BE was used as an internal standard for quantitation of BE in equine urine samples. The following ions were monitored: for BE *m/z* 240 (used for quantitation), 256, 361, and D_3-BE *m/z* 243, 259, 364. The standard curve for BE in urine ranged from 5 – 300 ng/mL. In order to validate the method, two levels of controls prepared in naive horse urine were analyzed on different days (15 and 75 ng/mL).

Results: In this paper the results from analysis of horse urine samples collected at four race tracks in the Greater Chicago Area between July 1, 2004 and June 30, 2005 are presented. During that period of time a total of 15 samples (0.16%) were reported positive for BE, five collected from thoroughbred and ten from harness horses. Out of 15 samples, three were reported positive without BE quantification (July 2004 to February 2005) and none of the estimated concentrations exceeded 25 ng/mL. The concentrations of BE in the remaining 12 samples ranged from 5 – 57 ng/mL. The limit of quantitation for BE was 5 ng/mL and the limit of detection was 1 ng/mL. The intra-day accuracy and precision for the low control was 2.8% and 20.6%, respectively, and for the high control urine preparations 2.2% and –2.3%, respectively. The inter-day accuracy and precision for the low controls was 10.4% and 9.5%, respectively, and for the high controls was 6.8% and 2.9%, respectively.

Conclusions: None of the BE concentrations reached or exceeded the threshold level of 150 ng/mL. The authors then postulate that these low concentrations found in urine are most likely a result of the external contamination and not premeditated cocaine administrations to horses.

Race Horses, Benzoylecgonine, GC-MS

K51 Utilization of a Pyroprobe Coupled to GC/MS in Drug Analysis and Toxicology

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The goal of this presentation is to illustrate the potential of pyrolysis as a tool for detecting biomarkers of abused drugs. This presentation will discuss the usefulness of a pyroprobe in detecting pyrolytic products of single drugs as well as mixtures of cocaine and methamphetamine. Attendees will become familiar with potential applications of pyrolysis to toxicology as well as understand the effects of varying experimental conditions and how they may alter the resulting pyrolytic products.

This presentation will impact the forensic community and/or humanity by demonstrating how pyrolysis coupled to GC/MS has the potential to model metabolism, therefore, with this method, a broad range of drugs may be analyzed in order to quickly detect metabolites that can be used in forensic laboratory analysis. This technique may be a potential tool to complement metabolic studies.

Smoked illicit drugs are of interest in forensic toxicology because smoking may produce unique biomarkers as a result of metabolism. Metabolic conditions can be partially modeled via pyrolysis, a process that decomposes a chemical compound by extreme heat. A pyroprobe is a thermal preparation device used to heat samples at high temperatures in order to breakdown the compounds into oxidation products. The pyrolytic products are then introduced into a gas chromatograph coupled to mass spectrometry (GC/MS) for identification. The present work employed a pyrolysis experiment with a pyroprobe coupled to a GC/MS. Advantages of this analytical technique include rapid sample analysis (on the order of 30 minutes) and minimal sample preparation. Pyrolysis has been used in forensic science for analyzing fibers, paints, photocopier toners and polymeric material. However to date, pyrolysis has not been used widely for toxicological research. This project will focus on the analysis of cocaine and methamphetamine and more generally, potential applications of pyrolysis to forensic toxicology. Pyrolysis has been previously carried out by heating an aluminum boat in a reference pan or by using an apparatus to simulate smoking of a tobacco cigarette laced with the analyte drug. Using such techniques, the primary pyrolytic product of cocaine is anhydroecgonine methyl ester (AEME) and methamphetamine is 1-phenylpropene, respectively. These pyrolytic products have been analyzed using both high performance liquid chromatography (HPLC) and GC coupled to MS. However, no research has been directed at simulating the metabolic conditions by pyrolysis. The ability to differentiate between inhalation via smoking versus exposure by an alternative method of ingestion is useful to the investigatory information. This study focused on the more commonly smoked drugs, cocaine and methamphetamine, along with the addition of certain cutting agents including lidocaine, caffeine, mannitol, starch and dextrose. Data obtained by pyrolysis was compared to the products from metabolized cocaine and methamphetamine reported by literature. The goal was to correlate degradation via pyrolysis to metabolic degradation as was feasible and appropriate. Several such correlations were identified and will be discussed. The effects of each of the following conditions were also studied:

1) Mixing cocaine and methamphetamine in various alternating ratios.
2) Altering methanol and ethanol as solvents.
3) Varying pyrolysis temperatures and GC conditions.

Pyroprobes, GC/MS, Toxicology

K52 Exemplification of Continuous Quality Improvement by Quality Surveillance: Laboratory Incidents and Corrective/ Preventive Approaches

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After attending this presentation, attendees will be acquainted with examples of laboratory incidents that could be used as a basis to improve performance of a laboratory by taking incidence-driven corrective/preventive measures.

This presentation will impact the forensic community and/or humanity by exemplifying incidents that are commonly encountered in a laboratory. Upon the rectification of those incidents by taking appropriate corrective/preventive measures, the overall performance of a laboratory would be improved. Monitoring of laboratory incidents is a realistic and simple approach for quality surveillance, thereby for continuous quality improvement.

The Federal Aviation Administration's Civil Aerospace Medical Institute (CAMI) conducts toxicological evaluation of postmortem biological samples collected from victims involved in fatal civil aircraft accidents. The submitted samples are analyzed for the presence of primary combustion gases, alcohol/volatiles, and drugs. Throughout the entire evaluation process, a high degree of quality control/quality assurance (QC/QA) is maintained, and continuous quality improvement is always administratively sought.

Under this philosophy, as quality surveillance, an "Incident Reporting" module was instituted in the CAMI Toxicology Database in October of 2000. Any member of the CAMI Laboratory was allowed to report an incident, but it was evaluated by designated QC/QA scientists on an incident-by-incident basis. This process involved (i) categorization of types and severity of incidents, (ii) best-educated estimates of dollar amounts and labor hours ($20.00/hour) associated with the incidents, and (iii) corrective/preventive measures taken in response to those events. Incidents with a labor hour of < 0.5 were not included. To evaluate effects of the reporting on the laboratory performance, the Toxicology Database was searched for incidents that were reported during 2000–2004. Associated dollar amounts/labor hours and types/severity of incidents were retrieved from the Database. Information related to the corrective/preventive actions taken to rectify the incident-related deficiencies was also collected.

These findings revealed that incident types pertained to accessioning, analytical, clerical, procedural, report generation, security, and other deficiencies. Severity of incidents, categorized as major, moderate, minor, and undefined, varied from analytical-batch rejection to typographical, to power outage. Corrective/preventive approaches included proofreading, counseling, and repeating tasks. This aspect also included implementing modified or new procedures and providing training to the laboratory members. Taking these quality approaches reduced the number of incidents from 61 in 2001 to 8 in 2004, thereby reducing the laboratory cost from $4,400 in 2001 to $730 in 2004. The decrease in labor-cost hours was consistent with the decrease in the incidents and dollar cost. Clerical errors were the highest in number, followed by analytical and accessioning. Although incident severity was highly prevalent in 2001, the overall severity decreased during ensuing years. Major incidents were associated with analysis, followed by accessioning, which is consistent with the very nature of postmortem forensic toxicology since these are essential components of a toxicology laboratory. Based upon the incident reporting, corrective/preventive measures—such as peer review, proofreading, procedure modification, and new method implementation—were undertaken. Training through mentorship, attending workshops/meetings/symposia, and taking courses was also provided to the laboratory members. These approaches led to a decrease in incidents during the period, 2002–2004. For example, there was a drastic decrease in clerical errors—no such incidents were significant enough to warrant corrective measures after 2002. Average completion time per case decreased from 46 days in 2003 (199 cases) to 35 days in 2004 (180 cases) for positive cases and from 37 days in 2003 (283 cases) to 31 days in 2004 (269 cases) for negative cases, indicating a tendency in the decrease in case completion time.

Findings from this study suggested that the quality surveillance improved product quality, saved time and money, streamlined and implemented procedures, thus enhanced the overall performance of the laboratory. The "Incident Reporting" will continue to be an effective and important aspect for improving quality of laboratories.

Toxicology, Laboratory Incidents, Continuous Quality Improvement

K1 Determination of Toxins and Alkaloids Markers of the Toxic Plant, *Ricinus communis Linn* by New Complimentary Technique

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Attendees will be briefed on the tentative identification of an unknown toxin protein in trace amounts by the presented identification scheme. The method is quick and efficient, especially for forensic samples.

Bioterrorism is a global threat. The appearance of some plant toxins in the terrorism literature has diverted the attention of the forensic science community to study these plant toxins, which are potential mass homicide agents. The author's research findings will impact the forensic community and/or humanity by helping forensic science and law enforcement agencies in the rapid identification and characterization of suspected plant toxin proteins.

The present paper describes the toxicological aspect of a plant having active principles in the form of alkaloids, glycoprotein's, etc. The toxicological study of this plant is very important for forensic science due to its appearance in terrorism literature and its potential for use as a mass homicide agent.

Goal: To develop a new complementary technique and protocol for the general protein and the identification of toxins derived from the seeds of plant r*icinus communis Linn.*

Method: The author used boiling methanol for the extraction of the active principles from a forensic sample suspected to contain caster seeds along with a reference sample of caster seeds collected from trans Himalayan and Himalayan region. Extracted residue was tested for its greatest solubility in different solvents. The author experimented with different percentages of SDS page and finally approved 12.5% SDS page for isolation, characterization, and tentative identification of the unknown toxin protein.

Results: The study reveals that the suspected samples and reference sample exhibits 8 different bands, visualized by using the Coomassie bright blue. The molecular weight of 8 protein bands was determined by using molecular dynamic image quant and the molecular mass of different 8 bands is started from 13 kd to 44 kd. The molecular weight of the three major bands no. 6, 7, & 8 is 23 kd, 20 & 18 kd. Bands no. 6, 7, & 8 is 23 kd, 20 & 18 kd, exhibits the agglutination & haemolysis activity in red blood of corpuscles.

Conclusion: The tentative identification of unknown toxin protein in trace amounts is possible by the presented identification scheme. The present method is quick and efficient especially, for the determination, identification, and characterization of plant toxin proteins in forensic samples.

Ricin, Toxin, Protein

K2 Mepivacaine Fatality Occurring After Local Anesthesia Was Administered Intravascularly During a Pre-Operative Procedure

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After attending this presentation, attendees will understand why qualified anaesthesiologists should administer the drug mepivacaine in order to avoid accidental death following intravascular infusion of the drug.

This presentation will impact the forensic community and/or humanity by showing the forensic community how fatal a local anaesthetic block of mepivacaine could be when accidentally administered intravascularly.

This is the case of a 92-year-old woman who was scheduled for surgery after falling at her residence and being diagnosed as having a left hip fracture. She had a recorded history of chronic pulmonary emphysema, congestive heart failure, coronary artery disease and glaucoma. While being prepared for surgery, she received an L3 in one block with 35 ml of 1.5% mepivacaine and sedation with midazolam. Five minutes thereafter, she had a witnessed cardiac arrest. Advanced Cardiac Life Support (ACLS) protocol was initiated with no success. At the autopsy, the descendent appeared relatively healthy and no trauma was found to have caused her death. Biological fluids and tissues were tested for basic, acidic, and neutral drugs using GC/MS.

In the postmortem heart blood, toxicological analyses identified *mepivacaine* at a concentration of 9.50mg/L, which is consistent with an intravascular administration. Vitreous humor, bile, liver, and brain specimens contained: 0.96mg/L, 0.46mg/L, 14.58mg/kg, and 2.18mg/kg mepivacaine, respectively. Atropine, levorphanol, pseudo/ephedrine, citalopram, dextromethorphan, lidocaine and midazolam were also present.

Administration of an appropriate dose of local anesthetic appears to be the single most important factor in preventing catastrophic reactions (*New England Journal of Medicine*, 295, 1397-1399, 1976).

The medical examiner ruled that the cause of death was mepivacaine toxicity and the manner of death was determined to be accidental.

Mepivacaine, Intravascular, Gas Chromatography/Mass Spectroscopy

K3 Plasma and Urine Amphetamine Levels Following Administration of Dexedrine®

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Attendees will gain knowledge of the absorption and excretion profile of amphetamine following administration of a typical dose of Dexedrine®.

This presentation will impact the forensic community and/or humanity by showing the plasma and urine concentrations following administration of 10 mg of Dexedrine®, a commonly used treatment for ADHD. This information will allow members of the community to assess unknown samples in light of these to help interpret findings.

Dexedrine® (d-amphetamine) has been used for many years for a number of clinical indications including narcolepsy, attention deficient disorder with hyperactivity (ADHD), and as a short term adjunct to a weight reduction program. It also has a long history of abuse. Use of stimulant medications for the treatment of ADHD has increased dramatically in the last few years as the number of patients diagnosed with this disorder increased and those diagnosed during childhood continued treatment well into their adult lives. Evaluation of urine concentrations of amphetamine following administration of Adderall® (another commonly prescribed form of amphetamine used for the treatment of ADHD) has previously been reported. No data currently exists on the excretion profile and plasma concentrations of amphetamine following typical therapeutic doses of Dexedrine®, thus the current study was initiated to describe urine and plasma profiles.

Subjects were administered 10 mg of d-amphetamine in the form of two 5 mg Dexedrine® tablets. Blood samples were collected in lithium heparin tubes prior to administration of the drug and at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 24, 36, and 48 hours following drug administration. Plasma was then separated from the sample and stored at ? 20ºC prior to analysis. Urine samples were collected in standard urine containers and stored at ? 20ºC prior to analysis. Samples were collected ad lib from each of the subjects prior to administration of the drug and at each urination for five days following initiation of the study.

Samples were analyzed using GC-MS following extraction of the analytes and derivatization with heptafluorobutyric anhydride (HFBA). Plasma samples were extracted using solid phase extraction of a 1 mL aliquot with United Chemical Technologies (UCT) XTRACKT, XRDAH203 high-flow 200 mg columns using a Zymark RapidTrace®. Urine samples were extracted using liquid-liquid extraction of 2 mL sample aliquots.

Urine samples were positive ($\geq$ 500 ng/mL) for no more than 48 hours following administration of the drug. The peak concentration of amphetamine seen in urine was 6,373 ng/mL. Plasma samples showed a peak concentration of 28 ng/mL and no samples contained detectable amphetamine (LOD 4 ng/mL) at 48 hours post dose. Amphetamine was detectable (LOD 5 ng/mL) in the urine up to 118 hours post dose.

Amphetamine, Plasma, Urine

K4 Quantitation of Propane in Biological Materials by Headspace GC

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Attendees will be briefed on an important technique to quantify propane and various information about propane poisoning.

This presentation will impact the forensic community and/or humanity by demonstrating one of the most difficult methods to confirm and quantify propane. The authors tried to determine the propane in biological samples.

Two persons died from a LPG explosion in an apartment and forensic quantification of propane, the predominant component of LPG, in the biological materials of the deceased was performed using headspace-GC/FID. Because of the variation of instrument performance and sample injection, the internal standard method was adopted. The stability, retention profile, xenobiotic, and similarity of partition coefficient were considered to select the appropriate internal standard and pentane in iso-butanol was chosen. Injecting a calculated volume of pure propane gas into a capped vial containing 2 mL of blood and 5 uL of internal standard created calibration standards. The calibration curve was linear from 0.09 ug/mL to at least 90.0 ug/mL. The method validation data of repeatability, recovery and linearity were also determined. The propane quantities in blood, fat, and brain tissue were calculated between 0.27 and 70.91 ug/mL (ug/g), and the maximal value was observed in fat. The confirmation of propane was conducted by solid phase micro-extraction followed by mass spectrometry.

Propane, Quantification, SPME GC-MS

K5 A Quetiapine-Linked Fatality

Patricia C. Studts, BS, Henry C. Nipper, PhD, and Richard Baltaro, MD, PhD, Creighton University Medical Center, Forensic Toxicology Laboratory, 601 North 30th Street, Omaha, NE 68131*

The goal of this presentation is to present a case of quetiapine overdose which resulted in a fatality and review the pharmacokinetics and adverse reactions to this drug, which has been assumed to be relatively non-toxic.

Few case reports of fatalities involving this drug have been published. This report will impact the forensic community and/or humanity by adding to the literature, and may assist forensic toxicologists and others in interpreting similar findings.

A 37-year-old white male with a history of depression was found dead at home. His current medications were listed as quetiapine, buspirone, and sertraline. Investigators estimated that approximately 96 quetiapine tablets were not accounted for, including one new bottle of 30 (300 mg) tablets. The older bottles contained 200 mg tablets. The Forensic Toxicology Laboratory received urine, blood, and vitreous humor. Cocaine and its metabolites, quetiapine metabolite, and nicotine were detected in the urine by GC-FID/NPD and by ion-trap GC-MS. Ethanol was also found in the urine by an ADH enzymatic assay. Blood ethanol was 0.029 g/dL. In the heart blood, quetiapine was 32,100 ng/mL, cocaine was 53 ng/mL, and benzoylecgonine was 819 ng/mL. The quetiapine and cocaine quantitations were performed at MedTox Laboratories.

Quetiapine (Seroquel) is classified as an "atypical" antipsychotic drug. Baselt (1) describes it as "a dibenzothiazepine derivative developed in 1993 for use as a neuroleptic agent." Its defined daily dose is 400 mg/d, has a half-life of 5-8 hours and is typically found in blood in the range of 195-632 ng/mL when used therapeutically (1).

The metabolite found in the decedent's urine is only one of approximately 20 metabolites of the parent drug. Metabolic pathways include sulfoxidation, carboxylic acid formation on the ethoxyethyl side chain, as well as hydroxylation in the 7 position. The 7-hydroxy metabolite does not apparently have significant pharmacologic activity.

According to the PDR (2), the clinical trial databases reported 6 overdoses with ingestions ranging from 1200–9600 mg with no fatalities. The 9600 mg overdose was associated with hypokalemia and first-degree heart block. Mortality cited for overdose of hospitalized patients is cited as 0.5% for the neuroleptic class of drugs (3). Overdose of these drugs is cited as similar to TCA overdose, but less toxic. So-called 'atypical' agents cause ECG abnormalities, but other case reports allege that quetiapine was less toxic than other atypical antipsychotic drugs. One series reports seizures, hypotension, QTc prolongation, and sedation to the point of requiring mechanical ventilation, similar to those effects seen with clozapine and olanzapine overdose (4).

Plasma concentrations, unhelpful in clinical management, may be useful in postmortem considerations. Dart (3) cites typical therapeutic concentrations of quetiapine of 190-630 ng/mL overlapping considerably with lethal blood concentrations of 240-4000 ng/mL. Postmortem redistribution, if any, remains unreported at this time for quetiapine.

References:

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3) Dart, Richard C., editor, Medical Toxicology, 3rd edition, Philadelphia, PA, Lippincott, Williams & Wilkins, 2004.
4) Trenton, A, Currier, G, Zwemer, F., Fatalities Associated with therapeutic use and overdose of atypical antipsychotics, CNS Drugs, 2003; 17(5): 307-324.

Quetiapine, Fatality, Overdose

K6 Relationship of Methamphetamine (MAP) Levels to Causes and Manners of Death in MAP-Related Casualties

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Diverse psychotic behaviors induced by MAP could be recognized by the MAP level in blood and urine by pharmacokinetics and manners of death so as to predict the psychotic behaviors before casualty. The goal of this presentation is to present a pilot designed to determine whether

toxicological profiles of the decedents' body fluids could be used to implicate the status of mood at the moment of death.

This presentation will impact the forensic community and/or humanity by presenting results, which suggest that the toxicological profiles are better related to patterns of death than manner of death. The findings may enable better utilization of the toxicological profiles in future judgment of forensic parameters including the cause and time of death.

Illicit drug abuse of MAP is a worldwide problem, and has caused a serious social crisis in the Taiwan community. MAP-induced fatalities with a high homicide rate (20-30%) are much higher in comparison with opiate-related fatalities' low homicide rate (0-5%). MAP is a psychostimulant and long-term MAP abusers may become addicts demonstrating psychosis, self-destructive behaviors, emotional disturbances, and schizophrenia-like behavior (MAP psychosis). MAP can induce long-lasting deficits of the innervations in the striatum from dopamine neurons of the substantia nigra. Diverse psychotic behaviors induced by MAP could be recognized by MAP level in blood and urine by pharmacokinetics and manners of death so as to predict the psychotic behaviors before the casualty. A pilot study was designed to determine whether toxicological profiles of decedents' body fluids could be used to implicate the status of mood at the moment of death. High blood/urine ratios can be associated with acute MAP use, a short period of time after MAP intake, and a manic emotional status. In comparison, a low blood/urine ratio can be associated with chronic MAP use, a longer period of time after MAP intake, and a depressive emotional status. A retrospective review of 586-MAP related fatalities collected from Forensic Medicine Center and Institute of Forensic Medicine in Taiwan, which had MAP levels in either blood or urine that were greater than 0.10 mg/L, found 88 cases with positive impressions of the causes and manners of death (3 unknown manner of death are excluded). Distinct patterns of MAP levels were found to be associated with a unique manner of death. Higher MAP concentrations were found in blood than in urine when death occurred shortly after an overdose of MAP that was linked either to accidental overdose (7.75 ± 1.99 μg/ml blood, 17.24 ± 4.27 μg/ml urine and 2.77 ± 1.04 blood/urine ratio; n=27) or to intentional suicide (15.71 ± 7.23 μg/ml blood, 13.86 ± 1.6 μg/ml urine and 1.23 ± 0.62 blood/urine ratio; n=4). Lower MAP blood levels and blood/urine ratios were found in cases of deaths by accidents (0.33 ± 0.09 μg/ml blood, 4.83 ± 1.89 μg/ml urine and 1.64 ± 1.05 blood/urine ratio; n=13) and suicides (0.77 ± 0.49 μg/ml blood, 6.02 ± 1.83 μg/ml urine and 0.43 ± 0.19 blood/urine ratio; n=9) not by caused MAP toxicity, making an influence of MAP mediated through depression and psychotic behaviors highly suspect. Much lower MAP blood/urine ratios were found among casualties of natural (0.40 ± 0.13 μg/ml blood, 18.56 ± 6.73 μg/ml urine and 0.38 ± 0.23 blood/urine ratio; n=12) or homicidal causes (1.07 ± 0.24 μg/ml blood, 10.56 ± 1.96 μg/ml urine and 0.14 ± 0.03 blood/urine ratio; n=23), suggesting that these deaths were relatively unaffected by the lower blood level of MAP. Chronic MAP abusers with low blood and high urine MAP levels appear to provoke violent behaviors resulting in the homicidal fatalities, and a relationship to amphetamine (AMP)-like psychosis is postulated. These results suggest that the toxicological profiles are related better to patterns of death than manner of death. The findings may enable better utilization of the toxicological profiles in future judgment of forensic parameters including the cause and time of death. (Supported by NSC 82-0412-B-016-075; 83-6016-F-096).

Methamphetamine, Manner of Death, Drug Concentrations

K7 Illicit Drug Related Fatalities in Taiwan During 1991-2003

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The goal of this presentation is to understand the epidemiology of illicit drug abuse in Taiwan during 1991-2003.

This presentation will impact the forensic community and/or humanity by providing data which supports that MAP-induced toxicity is closely related to the violent and destructive behaviors of MAP abusers.

Methamphetamine (MAP) and narcotics are considered two major illicit drugs that have resulted in serious social problems in Taiwan and other parts of the world. In addition to illicit drugs of narcotics-related substances (57.8% including opiate, morphine, and heroin), MAP constitutes the majority (41.8% including MAP semifinished material, MDMA and cannabis) of illicit drugs seized by the Investigation Bureau, Ministry of Justice and the National Police Administration (Taiwan 2003). By the end of 2003, violation of the Laws for the Control of Narcotics and the Laws for the Control of Illicit Substance constituted 16,013 cases in prison, which represented 39% of the 41,245 prisoners in Taiwan. This retrospective study of illicit drug-related decedents is proposed to understand the characteristics of MAP-related and narcotics-related fatalities by analyzing toxicological profiles, sex, age, and manners of death. During 1991 to 2003, illicit drug-related cases compromise 1,145 out of 14,887 forensic autopsy cases (7.7%) collected from the Institute of Forensic Medicine (Taiwan). MAP-related, narcotics-related, and multi-drug-related fatalities (constitute both MAP and narcotics-related substance in blood fluid) represent 371 (44%), 295 (35%) and 175 (21%) of the forensic autopsy cases, respectively. The mean age (average 30.1 ± 1.7 years old) of MAP-related, narcotics-related, and multi-drug-related was 32.1 ± 2.0, 30.2 ± 1.5 and 28.1 ± 1.7 years old. Males predominated (average 75%), MAP-related (73%), narcotics-related (78%), and multi-drug related (74%) fatalities. Manners of death of 371 MAP-related fatalities during 1991-2003 of natural, accidental, homicidal, suicidal, and unknown cause are 13%, 46%, 20%, 15%, and 6%, respectively. Manners of death of 295 narcotics-related fatalities during 1991-2003 of natural, accidental, homicidal, suicidal cause and unknown cause are 9%, 76%, 5%, 6%, and 4%, respectively. Mean concentration of MAP in blood and urine of MAP-related fatalities are 4.75 ± 0.73 mg/L and 17.38 ± 2.81 mg/L, respectively. Mean concentration of morphine in blood and urine of narcotics-related fatalities are 0.50 ± 0.06 mg/L and 8.39 ± 1.45 mg/L, respectively. Whereas higher and lower than 3 mg/L MAP concentration of blood indicated an over-dosage of illicit drug directly related to the accidental and suicidal causes of death and homicidal cause, respectively. The percentage of homicidal cause of MAP-related fatalities (20%) is higher than that of narcotics-related fatalities (5%). In conclusion, this data supports that the MAP-induced toxicity is closely related to the violent and destructive behaviors of MAP abusers.

Illicit Drug, Methamphetamine, Narcotics

K8 A Postmortem Distribution in a Fatal Case of *o*-Dichlorobenzene Poisoning

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After attending this presentation, attendees will have information about the distribution of *o*-dichlorobenzene and its metabolites 2,3-dichlorophenol, and 3,4-dichlorophenol in biological fluid and several tissues in a case of fatality due to *o*-dichlorobenzene.

This presentation will impact the forensic community and/or humanity by showing an unusual dichlorobenzene poisoning case and the distribution in various tissues.

O-dichlorobenzene has been used as a solvent, insecticide, and a degreasing agent. An accurate and simple method was developed to determine dichlorobenzene and its metabolites, dichlorophenols, in biological fluid and tissues by using gas chromatography/mass spectrometry (GC/MS) with solid phase microextraction (SPME). For analysis of dichlorobenzene, an assembly of SPME with a replaceable extraction fiber, coated with 100mm polydimethylsiloxane, was used with a headspace technique. SPME fiber, coated with 85mm polyacrylate, was used to analyze dichlorophenols with an immersion technique. The calibration curves showed good linearity at 0.99 in the range of 20 to 400mg/mL for both techniques.

A male age 34 with schizophrenia was found dead. Toxicological analyses to identify and quantify *o*-dichlorobenzene and dichlorophenols were performed on blood and tissues taken at autopsy. The concentrations of *o*-dichlorobenzene were 39.9mg/mL (blood), 89.3mg/g (spleen), 63.1mg/g (lung), 50.6mg/g (kidney), 90.6mg/g (brain), 298.5mg/g (heart), and 101.4mg/g (liver). Its metabolites, 2,3-dichlorophenol and 3,4-dichlorophenol concentrations were 2.09 and 1.65mg/mL (blood), 3.53 and 2.69mg/g (spleen), 3.30 and 3.33mg/g (lung), 7.41 and 8.02mg/g (kidney), 1.13 and 0.73mg/g (brain), 1.81 and 1.38mg/g (heart), 6.44 and 4.78mg/g (liver), respectively.

o-Dichlorobenzene, SPME, Dichlorophenol

K9 MDMA in Four Medical Examiner's Cases in the City and County of San Francisco

Nikolas P. Lemos, PhD, Steven B. Karch, MD, Elin Lin, MS, Glenn Nazareno, MD, Venus Azar, MD, Jon Smith, MD, Amy P. Hart, MD, and Boyd G. Stephens, MD, Office of Chief Medical Examiner, Hall of Justice, North Terrace, 850 Bryant Street, San Francisco, CA 94103*

The goal of this presentation is to alert the general community on the apparent risks of MDMA use and will also aid forensic toxicologists in the interpretation of postmortem and antemortem MDMA levels.

This presentation will impact the forensic community and/or humanity by alerting the general community of the apparent risks of MDMA use and will also aid forensic toxicologists in the interpretation of postmortem and antemortem MDMA levels.

3,4-methylenedioxymethamphetamine (MDMA or 'ecstasy') is a 'psychedelic amphetamine' tied to the underground rave and dance club scenes throughout the world, but is also being considered for use by therapists as an adjunct agent. The Office of the Chief Medical Examiner for the City and County of San Francisco serves a population of approximately 750,000 and this number has remained stable for several decades. In 2002, 1,463 cases came under the jurisdiction of the SFOCME; MDMA was detected in blood, urine, and/or tissue of four cases, giving an incidence of 0.5 per 100,000 people. The data presented herein is the result of a retrospective analysis of all death investigations carried out by the SFOCME, from January 1, 2002 until December 31, 2002. The median age of decedents was 22 years (SD=3, range 18-25 years). Decedents were overwhelmingly male (75%) and mostly black (50%). Gunshot wound was the cause of death in two cases, and asphyxia due to hanging in one. In only one instance, a case of anoxic-ischemic encephalopathy secondary to acute MDMA intoxication was MDMA actually considered the cause of death. Benzoylecgonine was detected in the urine of one, and dextromethorphan was detected in the blood and urine of the second of the two decedents who died due to gunshot wounds. Amphetamines were screened in the biological specimens of these cases using EMIT. MDMA and other amphetamines were then extracted from biological specimens using liquid-liquid extraction for alkaline drugs using reconstitution in chloroform, derivatization with acetic anhydride, and reconstitution in methanol prior to identification and confirmation/quantitation by gas chromatography–mass spectrometry (GC-MS) in the electron impact ionization mode. In three of the four cases, where death was immediate (i.e. gunshot wounds and asphyxia due to hanging) the mean MDMA postmortem femoral blood concentration was 0.30 ± 0.07µg/mL and the mean postmortem urine concentration was 13.1 ± 7.6 µg/mL. In the case where death was actually due to MDMA intoxication, the antemortem serum MDMA concentration was 0.7 µg/mL near the time of admission, falling to 0.3 µg/mL seven and a half hours later; the respective antemortem serum MDA concentrations were 0.02 µg/mL, and 0.01 µg/mg. Both MDMA and MDA were present in the antemortem urine specimen (at concentrations of 10.4 and 0.45 µg/mL, respectively). For comparison, a review from another Medical Examiner's Department where a 24-year-old white male died of acute polysubstance toxicity involving alcohol, cocaine, heroin, and MDMA, where the postmortem MDMA blood concentration was 1.7 µg/mL and the postmortem MDA blood concentration was 0.14 µg/mL. The expectation is that this study will alert the general community on the apparent risks of MDMA use and will also aid forensic toxicologists in the interpretation of postmortem and antemortem MDMA levels.

MDMA, Ecstasy, Postmortem

K10 Methadone Related Deaths in the City and County of San Francisco

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The goal of this study was undertaken to determine whether there had been any change in the incidence of methadone related deaths, as either the principal cause of death, or as a contributing factor, since the publication of an earlier report in 1999.

This presentation will impact the forensic community and/or humanity by demonstrating that despite a continuing increase in the amount of methadone prescribed, and increased concerns about methadone diversion and toxicity, neither the demographic profile, nor the rate of methadone related deaths in the City and County of San Francisco have changed since 1997. Whether this is due to changes in either prescribing and clinical care or other unique features of drug takers in San Francisco is, at this time, impossible to say.

Goal: Public concern about methadone diversion and the accidental fatalities that may result is a cause of great concern. In spite of extensive media attention, however, the true magnitude of the problem is not known with any certainty, and the frequency of the problem seems to vary widely from city to city. The SFOCME serves a population of approximately 750,000, and this number has been stable for several decades. In 1999

records were reviewed for all deaths occurring in the City of San Francisco, from 1997 through 1998, where methadone was detected in blood or urine samples (West J Med. 2000 Jan;172(1):11-14). This new study was undertaken to determine whether there had been any change in the incidence of methadone related deaths, as either the principal cause of death, or as a contributing factor, since the publication of an earlier report.

Design: Retrospective analysis of all death investigations carried out by the San Francisco Office of the Chief Medical Examiner, from January 1, 2002 until December 31, 2002.

Findings: In 2002, 1,463 cases came under the jurisdiction of the SFOCME office; methadone was detected in blood or urine of 35 cases, giving an incidence of 4.4 per 100,000 compared to a rate of 5.0 per 100,000 in the 1997-1998 study (presuming a constant population base of 750,000). The median age of decedents was 44.9 years (SD=10.4, SE = 1.8, range 23-61 years). Decedents were overwhelmingly male (85%), and predominantly white (66%). In 2002, death was attributed to trauma or underlying medical disorder in 24 (72%) of cases. In the remaining nine cases the diagnosis was given as poly-drug abuse (6), or methadone. Cocaine was present in five of the nine cases, methamphetamine in two, and morphine in three. In each of the three cases where morphine was detected, cocaine was also present. The rate for co-abuse of cocaine was slightly higher than in an earlier study, and the rate for morphine use was slightly lower, but the small sample size precludes definite conclusions. Individuals dying of methadone toxicity were significantly younger than individuals where presence of the drug was an incidental finding (42.0 vs. 45.8 years vs. 48.3 and 46.3 years in the first study), and suffered from fewer underlying disorders. Chronic illnesses, including alcoholism, HIV, and Hepatitis B and C infection, were common in the group where methadone was an incidental finding. The mean methadone blood concentration was 835 ± 170 ng/mL compared with a mean of 957± 140 ng/mL in the earlier study.

Conclusion: Despite a continuing increase in the amount of methadone prescribed, and increased concerns about methadone diversion and toxicity, neither the demographic profile, nor the rate of methadone related deaths in the City and County of San Francisco have changed since 1997. Whether this is due to changes in either prescribing and clinical care or other unique features of drug takers in San Francisco is, at this time, impossible to say.

Methadone, Epidemiology, San Francisco

K11 Analysis of Amphetamine and Methamphetamine in Whole Blood by Solid Phase Extraction and Gas Chromatography - Mass Spectrometry

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Attendees will learn an acceptable method for the solid-phase extraction of amphetamine and methamphetamine from blood matrix as an alternative to liquid/liquid extraction methods.

This presentation will impact the forensic community and/or humanity by demonstrating a solid-phase extraction procedure, which yields acceptable results, is time efficient (taking an average of 2.5 hours from start to completion), and requires a small amount of sample and limited amounts of organic solvents.

The purpose of this study was to demonstrate that the Cerex Polycrom Clin II solid-phase extraction column (SPEware, San Pedro, CA) provides acceptable extraction of amphetamine (AMP) and methamphetamine (MAMP) from whole blood. The Cerex Polycrom Clin II solid phase extraction (SPE) column is packed with a patented divinylbenzene polymer that is highly cross-linked and functionalized to perform in dual mode utilizing hydrophobic and cation exchange mechanisms.

The procedure requires pre-treatment of the blood samples (1 mL) with 2 mL of a phosphate buffer (pH 6), vortex mixing for 30 seconds, followed by sonication for 10 minutes, and then centrifugation for 6 minutes. Samples were then added to the SPE columns and washed with deionized water, pH 6-phosphate buffer, methanol, and ethyl acetate. During the wash procedures, the samples were vacuumed at 2-5 psi. The columns were dried at full vacuum for 3 minutes and then eluted with ethyl acetate containing 2% concentrated ammonium hydroxide. A solution of 1% HCl in methanol was added to the extracts, vortex mixed, and evaporated to dryness. The dried residues were derivatized with acetic anhydride and transferred to auto-sampler vials and analyzed by GC/MS (HP6890 GC, HP5973 MS) utilizing selected ion monitoring. Deuterated internal standards were used for the quantitation of AMP and MAMP. Ions monitored for the acetyl derivatives were (underlined ions are used for quantitation): AMP: $\underline{118}$, 44, 177; d_{11}-AMP: $\underline{128}$, 48, 188; MAMP: $\underline{100}$, 58, 191: d_{14}-MAMP: $\underline{107}$, 65, 205.

The linearity study exhibited an upper limit of linearity at 4000 ng/mL, and lower limit of detection and quantitation at 10 ng/mL for both analytes. Correlation coefficients were 0.9994 and 0.9993 for AMP and METH, respectively. Daily linear regression calibration curves for each analyte yielded correlation coefficients of 0.9995 or greater along the dynamic range. Carryover was not observed at 10,000 ng/mL. Extraction efficiencies at 100 ng/mL averaged 92% and 91% for AMP and MAMP, respectively. Precision was evaluated on three separate days at 50 ng/mL and 200 ng/mL with 5 replicates at each concentration. The within-run precision yielded average responses from 47 – 51 ng/mL (%CV 1.1 – 5.8) and 202 – 207 ng/mL (%CV 3.9 – 8.6) for AMP, and 47 – 55 ng/mL (%CV 1.3 – 4.6) and 199 – 205 ng/mL (%CV 3.8 – 8.7) for MAMP. The between-run precision for AMP produced CV results of 5.5% and 6.0% at the 50 ng/mL and 200 ng/mL levels respectively. The between-run precision for MAMP produced CV results of 7.3% and 6.1% at the 50 ng/mL and 200 ng/mL levels, respectively.

A small comparative study was conducted using this SPE procedure on preserved whole blood samples that had been previously analyzed by a liquid-liquid extraction method. Good agreement was observed between these two procedures. Correlation studies yielded correlation coefficients of 0.9732 for AMP and 0.9966 for MAMP. The results of the comparative study were analyzed statistically using a two-tailed Student's t-test. The calculated t values were 1.714 for AMP and 0.5068 for MAMP and the critical t values were 2.086 for AMP and 2.048 for MAMP at the 95% confidence level. The t-test indicates there is no significant statistical difference between the results from the two methods.

An interference study was conducted using a blank control and spiked blood samples at 50 ng/mL of AMP and MAMP. The following drugs were added to these controls at a concentration of 10,000 ng/mL: 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxy-N-ethyl-amphetamine, phenylpropanolamine, ephedrine, pseudoephedrine, propylhexadrine, fenfluramine, mecathinone, and p-methoxymethamphetamine. Phentermine, propylhexadrine, and fenfluramine were found to cause interferences with chromatography of the target analytes. If these drugs are anticipated, an alternative derivatization process, such as a fluorinated derivative, can be used to resolve these interferences.

This solid-phase extraction procedure yields acceptable results, is time efficient (taking an average of 2.5 hours from start to completion), and requires a small amount of sample and limited amounts of organic solvents.

Solid Phase Extraction, GC/MS, Methamphetamine

K12 The Death Pattern and Distribution of Toluene in Blood of Glue Sniffers

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After attending this presentation, attendees will have information about the types of death and the distribution of toluene following intoxication from inhalants.

This presentation will impact the forensic community and/or humanity by showing the death pattern and toluene blood concentration following intoxication from inhalants.

The blood toluene concentration was determined by using GC/MS with HS-SPME of postmortem blood, quantitatively. Fuel gases were analyzed using GC/FID with headspace technique in postmortem blood, qualitatively. Seventy-five cases of death associated with the inhalation of glue or fuel gases was reported in Korea over three years (1996-1998). In twenty-seven of the cases of death due to glue sniffing, nine persons died as a result of a fall while intoxicated and their blood toluene concentration was fairly high in the range of 1.3~21.6mg/mL (average 10.4mg/mL). However, nine persons who died suddenly due to glue sniffing showed low toluene blood concentration in the range of 0.5~22.6µg/mL (average 4.0mg/mL, only one case showed 22.6mg/mL, seven cases were below 2.0mg/mL). In cases of death due to fuel gas sniffing, fifty-four persons died of acute fuel gas inhalation or suffocation and six people who died due to sniffing fuel gases as well as glue.

Inhalation, Death Pattern, Glue-Sniffing

K13 A Suicide By Brake Fluid Ingestion

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The goal of this presentation is to present a case report on brake fluid intoxication and resulting death.

This presentation will impact the forensic community and/or humanity by demonstrating a rarely reported manner of death where the only significant toxicological findings were easily made using routine alkaline drug screen methods and instruments.

Ingestion of brake fluid is a rarely reported phenomenon; in fact, no case reports are present within the English literature. Numerous case reports of ingestion of antifreeze (ethylene glycol) and glass cleaner (ethylene glycol butyl ether) are present within the literature, which are similar chemical compounds to the glycol ethers present within brake fluid.

Both ethylene glycol and EGBE poisoning cause metabolic acidosis. In addition, EGBE causes central nervous system depression and hemolysis. Ethylene glycol causes oxalate crystal formation within the renal tubules and renal failure. Ethylene glycol and EGBE poisonings have been successfully treated with hemodialysis and ethanol infusion.

The authors report a case in which brake fluid ingestion was the MO in a suicide. The decedent was a 38-year-old Caucasian male who drank an unidentified amount of *Snap® Heavy Duty Brake Fluid* an unknown time before his death. The decedent had a previous history of suicide attempts, including a self-inflicted gunshot wound.

An autopsy was performed at the Bexar County Medical Examiner's Office. The autopsy revealed a normally developed, well-nourished, adult Caucasian male, 67 inches tall and weighing 154 pounds. There was no evidence of trauma. The internal autopsy was remarkable for 300 cc of malodorous oily fluid within the stomach, consistent with the brake fluid submitted with the body, marked pulmonary edema and mild hepatic steatosis. A full microscopic examination was performed and showed no abnormalities. Specifically, crystal formation within the renal tubules was not present.

Remarkable toxicology included the presence of several related glycol ethers in the blood and gastric contents. These compounds manifested in an alkaline drug extraction analyzed by GC flame ionization and subsequent GC/MS with electron impact ionization. They may include triethylene glycol monobutyl ether, diethylene glycol monobutyl ether, triethylene glycol monoethyl ether, and diethylene glycol monoethyl ether. Among others, the trade name Dowanol®, a product of the Dow Chemical company, is associated with these compounds. EI fragmentation patterns were confirmed by matching with similar eluting peaks from the remnants of the actual brake fluid container. Verification with pure standards was not pursued, as they are difficult to obtain. Ethylene glycol was notable by its absence in the blood.

Brake Fluid, Suicide, Gas Chromatography

K14 Fatal Chloroquine Intoxication in a 2-Year-Old Child

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After attending this presentation, attendees will have a better understanding of the signs and symptoms of chloroquine intoxication and the distribution of chloroquine in postmortem specimens from a child fatality.

This presentation will impact the forensic community and/or humanity by assisting forensic toxicologists and medical personnel to consider the possibility of chloroquine intoxication in a child with a previous history of malaria presenting with symptoms of uncontrollable shaking, profuse sweating, bradycardia, and diffuse cerebral edema.

The authors present the case history and toxicology findings of a child fatality involving chloroquine. A 2-year-old male was found shaking, gasping for air, and complaining of feeling hot shortly after eating his dinner. The child lost consciousness and was taken to the emergency room. Observed symptoms in the hospital included bradycardia, sweating, hypoxia, and diffuse cerebral edema. He died the following day. Few details regarding the case history were known at the time. The child had been previously treated for malaria in Africa before his family migrated to the U.S. less than a year prior. However, there was no recent history of illness or allergies, and prior to this incident the child had been described as a normal healthy 2-year-old.

Specimens were submitted for a full toxicological analysis, including an alcohol analysis by headspace gas chromatography with flame ionization detection; a screen for drugs of abuse and several prescription drug classes using an enzyme-linked immunosorbent assay technique (ELISA); and a screen for basic compounds using gas chromatography-mass spectrometry (GC-MS). Positive findings were confirmed and quantitated using GC-MS. Chloroquine was detected in subclavian blood at a concentration of 34.4 mg/L.

Chloroquine is used as an antimalarial agent. It is not available as an over-the-counter medication in the U.S. and it is suspected that the family brought chloroquine from Africa. Symptoms of chloroquine toxicity range from headache, confusion, dizziness, gastrointestinal upset, and visual disturbances, to hypotension, vasodilation, respiratory depression, and eventual cardiac arrest. The cause of death in this case was determined to be "*chloroquine intoxication,*" and the manner of death was "*accident.*" A discussion of the case circumstances, the autopsy and toxicology findings, and chloroquine pharmacokinetics will be presented.

Chloroquine, Fatality, Child

K15 Performance Characteristics of the Cozart® EIA Cannabinoids Microplate Kit for Oral Fluid in Comparison With GC-MS

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After attending this presentation, attendees will understand the analysis of cannabinoids in oral fluid by ELISA and GC-MS.

This presentation will impact the forensic community and/or humanity by providing information on the testing of cannabinoids in oral fluid and detailing the analysis of samples collected from individuals being monitored for drug use.

Goals: This project was carried out to evaluate the performance characteristics of the Cozart® EIA Cannabinoids microplate as a preliminary screening device for delta-9-tretrahydrocannabinol (Δ9-THC) in oral fluid.

Methods: Oral fluid samples (N=100) were collected from individuals being monitored within a drug treatment program and were screened according to the manufacturers instructions. Samples were collected using the Cozart® RapiScan collection system, which included a 1:3 dilution of the sample in a preservative buffer. All samples, calibrators (0, 6, 30, and 150 ng/mL equivalent in neat oral fluid) and controls (0 and 45ng/mL) were assayed in duplicate. Gas chromatography–mass spectrometry (GC-MS) confirmation for Δ9-THC was carried out on all samples. The LOQ/LOD for Δ9-THC was 3 ng/ml by GC-MS.

Results: Of the samples screened 75 screened positive and 25 screened negative, 73 were confirmed positive for Δ9-THC and 27 were confirmed negative by GC-MS. Concentrations of Δ9-THC ranged from the LOD of 3 ng/ml to greater than 1µg/ml. Sensitivity and specificity for the assay were 100% and 93% respectively.

Conclusions: The Cozart® EIA Cannabinoids Microplate Kit for oral fluid employing a 30ng/ml cut-off had good sensitivity and specificity with an overall assay agreement of 98% with GC-MS and provided adequate performance as a screening procedure for the identification of Δ9-THC in oral fluid.

Cannabinoids, ELISA, Oral Fluid

K16 Cocaine Related Deaths: An Enigma Still Under Investigation

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The goal of this presentation is to present to the forensic toxicology community several references to aid in the determination of cocaine related death.

This presentation will impact the forensic community and/or humanity by providing information about the interpretation of cocaine related deaths, which is still very difficult and disputed. Although literature offers many toxicological data about cocaine involving death, the correlation of a specific blood or tissue concentration with toxicity is not directly proportional to the height of these levels. Many factors make the interpretation of toxicological findings in cocaine associated deaths more complicated:

- fast absorption of drug assumed by "snorting" or "intravenous"
- different metabolism in chronic or occasional user
- *in vitro* degradation
- postmortem redistribution of cocaine and its major metabolite, benzoylecgonine
- different rates of crossing blood-brain barrier (benzoylecgonine crosses with greater difficulty)
- interactions of cocaine and alcohol or other drugs (such as disul firam, amitriptyline, procanamide, quinidine, vasoactive compounds, etc.)

As a consequence, many authors recommend caution in not misinterpreting toxicological data, especially by untrained and inexperienced operators, (not including real forensic toxicologists), because, with the exception of massive overdose (when the mechanism of death is perfectly clear), most cocaine related deaths occur in chronic drug users. Also, the cocaine concentration found in postmortem blood might not be representative of the concentration present at the time of death.

Isolated blood cocaine levels, without any other parenchymal distribution analytical data, cannot be used to explain the cause of death, because - for example - cocaine associated sudden death is not dose related.

Several studies have demonstrated that blood and brain ratios of cocaine/benzoylecgonine concentrations are greatly important to suggest a parameter to identify and discriminate death due to cocaine overdose from death where the presence of cocaine is simply an incidental finding.

Blood and brain levels used to determine cocaine and benzoylecgonine concentrations ratios are the best matrix for postmortem analytical researches, because even if cocaine blood concentrations change significantly after death, cocaine appears to be more stable in the lipid-rich tissue of the brain.

In Spielher and Reed's 1985 study, the interpretative value of the determination of cocaine and benzoylecgonine in brain tissue was investigated.

They found that in 37 autopsied cases of cocaine related deaths (overdose) the concentration of cocaine found in the brain is four to ten times higher than in the plasma; where cocaine was only an incidental finding (46 cases - instances of murder, accidental death, etc.), the average blood/brain ratio was only 2:5 for cocaine and 1:40 for benzoylecgonine. In the forensic toxicology division, all suspected cocaine cases (overdoses and incidental deaths) were investigated as to cocaine and its metabolites distribution.

The authors apply Spielher and Reed's model to cases performed during 1990 to the first six months of 2004 on 77 cocaine overdose fatalities, and 30 cases where cocaine was incidental to the cause of death.

Cocaine and benzoylecgonine were extracted by SPE and derivatized compounds were identified and quantified by means of a gas chromatography-mass spectrometry (GC/MS) using selected ion monitoring detection (SIM).

The findings were in agreement with those of Spielher and Reed. The authors found that in overdose cases the ratios of cocaine/benzoylecgonine in the brain was 10:28 and in the blood 0:69. These ratios were clearly different from those found in incidental cases (brain mean was 0:71 and blood mean was 0:21).

The brain/blood ratios of cocaine and benzoylecgonine concentration in overdose cases were found to be 8:06 for cocaine and 0:67 for benzoylecgonine; in incidental cases the ratios were 2:28 for cocaine and 1:67 for benzoylecganine.

In conclusion, brain tissue appears to be a good sample for the determination of cases of cocaine involved deaths. The brain concentration levels related to the blood provide useful information in the determination of overdose as compared to cocaine as an incidental finding.

Cocaine, Death, Brain

K17 Observations of Endogenous Levels of GHB in Urine Over Time

Negin Aghdaie, MS, LAPD - SID, 555 Ramirez Street, Space 270, Los Angeles, CA 90012*

The goal of this presentation is to provide the forensic toxicology community with information about the fluctuation of endogenous GHB levels in urine over time among different individuals.

This presentation will impact the forensic community and/or humanity by aiding forensic toxicologists with interpretation of urine GHB concentrations obtained in forensic casework. It will be made apparent that comparison of a background urine sample from an individual, with the forensic urine sample obtained from the same individual at the time of assault does not provide valuable information.

This poster will display the results obtained from the thesis research project completed as part of the Criminalistics MS program at California State University, Los Angeles. The focus of the project was to study the fluctuation of endogenous GHB concentration in human urine over time.

Gamma-hydroxy butyric acid (GHB) is a central nervous system depressant with hallucinogenic and euphoric effects. In a criminal context, it can be used along with alcohol for its incapacitating effects in drug facilitated sexual assaults. Forensic toxicologists commonly receive requests to analyze urine for the presence of GHB in sexual assault cases. The interpretation of the quantitative results in these cases can be ambiguous. This is due to the fact that GHB is an endogenous compound in human urine. It has also been demonstrated that the concentration of endogenous GHB varies between subjects. Therefore, it is essential to verify the amount of GHB found in urine as endogenous or exogenous in origin. To date, there is no widely accepted concentration threshold that distinguishes endogenous levels from ingested levels of GHB in urine.

Inter- and intra-individual variations of endogenous urine GHB levels were evaluated. The first goal of the study was to compare urine GHB concentrations between subjects. The second goal was to determine if an individual's endogenous GHB concentration is consistent over time. The establishment of a fixed endogenous GHB concentration level per individual would be valuable for forensic casework. The ability to compare the GHB concentration of a background sample obtained from a victim with the forensic sample, obtained from the same victim at the time of assault, would simplify the interpretation of the results.

In order to achieve its goals the study was divided into two parts. One hundred forty-seven urine samples from five individuals (non GHB users) were collected over a 30-day period and subsequently analyzed. During the first 48 hours of the study, an aliquot of every urine void from each subject was collected and analyzed. The second part of the study involved analysis of samples collected from the remaining 28 days, at which time only an aliquot of the daily morning first void from each individual was collected and analyzed. Fluctuations of endogenous urine GHB concentrations in both the two-day study as well as the month long period appear random in all participating individuals. No clear concentration pattern was observed. This implies that it is not feasible to try to establish a fixed background endogenous GHB level for any one individual.

The average GHB concentration among all individuals in the study was 3.2µg/mL. The highest concentration found among all samples was 9.8µg/mL. Several specimens in this study approached 10µg/mL, which some analysts consider a threshold level indicative of GHB ingestion. Findings of endogenous urine GHB concentration at such levels suggest necessary reassessment of 10µg/mL as the threshold level of endogenous urine GHB.

Toxicology, GHB, Endogenous

K18 Is Car Driving Under the Influence of Sauerkraut Punishable?

Beat Horisberger, MD, Patrice Mangin, MD, PhD, and Marc Augsburger, PhD, Institute of Legal Medicine, University of Lausanne, Rue du Bugnon 21, Lausanne, 1005, Switzerland*

After attending this presentation, attendees will understand that an alcohol level greater than 2 g/kg cannot be explained by an endogenous production and that one should not believe any allegation made by a driver under the influence of alcohol.

This presentation will impact the forensic community and/or humanity by demonstrating the utility of using any possibilities to find the real source of alcohol in blood in case of major alcohol-impaired driving.

The forensic expert must occasionally face issues that are not only unusual but also quite interesting on an anecdotal level, without ever compromising the underlying scientific validity of the expert's work. This case certainly belongs to this peculiar type of forensic investigations.

The case concerns a criminal investigation of a woman driver, aged 57 at the time of the events, and suspected of driving under the influence of alcohol. The driver was involved in a traffic accident, causing only minimal material damage. Her alcohol levels were measured at 2.3 g/kg, as determined by an ethylometer test conducted by the police after the accident. A blood test, carried out shortly after, revealed an alcohol level (2.16 g/kg) consistent with major alcohol-impaired driving.

The driver assured the investigators that she did not consume the slightest drop of alcohol. A medical certificate delivered subsequently by her treating physician indicated that the observed alcohol levels were caused by gastrointestinal fermentation of sauerkraut ingested prior to the accident. The physician's conclusions relied on evidence found in the scientific literature and on various tests carried out on his patient.

A forensic expert was mandated by the investigative magistrate to determine whether the woman driver was capable of producing ethanol endogenously and if so, to describe the circumstances and the magnitude of this phenomenon.

The goal of the forensic investigation was to determine whether sauerkraut consumption could indeed lead to alcohol levels above 2 g/kg, in which case the authorities should recommend that prior to driving, sauerkraut should only be "eaten in moderation."

Drunk Driving, Endogenous Alcohol Production, Sauerkraut

K19 Oxycodone Blood Concentrations in Seventy Postmortem Cases

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The goal of this presentation is to assist the forensic pathologist and toxicologist in evaluation of postmortem oxycodone blood concentrations.

This presentation will impact the forensic community and/or humanity by assisting forensic pathologists and toxicologists in assessment of the role of oxycodone in sudden and unexpected deaths.

The authors present the postmortem blood oxycodone findings in 70 deaths: 4 cases of fatal intoxication due solely to oxycodone (3 men, ages 22, 23, 46 yrs; 1 woman, 21 yrs); 38 cases of multiple drug intoxication involving oxycodone (22 men, mean age 37 yrs, ranging from 19 to 78 yrs, and 16 women, mean age 39 yrs, ranging from 20 to 61 yrs); 28 cases of natural causes of death where oxycodone was an incidental finding (19 men, mean age 35 yrs, ranging from 18 to 79 yrs, and 9 women, mean age 39 yrs, ranging from 35 to 63 yrs). Oxycodone was isolated from blood by solid phase extraction with n-butyl chloride/acetonitrile mixture. Acetyl-oxycodone derivative was prepared with acetic anhydride/pyridine and

analyzed by GC/MS with separation on a HP-5MS column (30m x 0.25mm id x 25 *u*m film thickness) at the following temperatures: initial, 60ºC; ramp, 20ºC/min; final 280ºC; with a retention time of 14.52 min for oxycodone and deuterated oxycodone (IS). Ions monitored in SIM mode for acetyl-oxycodone, and acetyl-d_3-oxycodone was 357,358,314 m/z and 360,317m/z, respectively. The calibration was linear from 0.10 - 2.0mg/L. Oxycodone blood values are given in Table 1.

Table 1.	N	Oxycodone Blood Mean, mg/L	(Range, mg/L)
Sole agent	4	0.50	(0.23 – 0.76)
Mixed drug	38	0.42	(0.06 - 1.6)
Natural	28	0.19	(0.10 – 0.6)

Acetaminophen (APAP) was present in 12 of the 38 deaths due to multiple drugs indicative of ingestion of oxycodone/APAP combination tablets. Commonly encountered drugs in the multiple drug deaths were; benzodiazepines, 13 cases; carisoprodol, 13 cases; cocaine, 8 cases; and antidepressants, 4 cases. In addition to oxymorphone, a metabolite of oxycodone, other opiates present were fentanyl, 3 cases and methadone in 2 cases. While tolerance is a major consideration in the interpretation of postmortem oxycodone concentrations, these data are consistent in that therapeutic blood values are expected to be less than 0.25 mg/L, and toxic or lethal blood concentrations would be expected to be greater than 0.40 mg/L.

Oxycodone, Fatal Poisoning, GC/MS

K20 Use of Tetrahydrozoline (Visine®) for Chemical Submission and Sexual Assault in Children

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After attending this presentation, attendees will have knowledge of the potential use of tetrahydrozoline as an agent for chemical submission and sexual assault.

This presentation will impact the forensic community and/or humanity by providing a better recognition of a drug potentially used for sexual assault. Without this knowledge this drug may go undetected in potential criminal cases.

This is a report of the use of a commonly available over-the-counter drug to induce an obtund compliant victim with no memory of the period during the sexual assault. It provides the results from a police investigation into crimes against children as well as the investigation of the method used by the perpetrators of the crimes to repeatedly allow assault while inhibiting any memory of the time under sedation. It will also discuss toxic mechanism of tetrahydrozoline.

In 2003, police investigation located pictorial evidence on the Internet of adults having sex with children. Further investigation located these children in the United States and they were removed from the home. An adult male relative with sole legal custody had primary care of the children and was a suspect in the investigation. The 4 female children were 2 years old through 8 years old at the time the abuses occurred. After being removed from the home the children were interviewed and entered into counselling. However, during all interviews and counselling sessions over the subsequent year following removal from the home the children denied having any specific memories of the sexual assaults, despite the pictorial evidence in which police could positively identify them. The children did give a history of being given a substance by their "father" when they were "bad," prior to their punishment, so that they "would not remember." The home of the male guardian was searched for drugs of abuse and those drugs known to be used in chemical submission. No drugs were located. Interviews with the wife of the suspect (not the mother of the children) indicated the suspect would give the children Visine® prior to any sexual assault and the suspect would routinely carry around a bottle of Visine® with him. This history was obtained more than one year after the children had been removed from the home and so prohibited any testing of the children for tetrahydrozoline. It is unclear where the suspect learned of sedative effects of Visine®.

Visine® contains 0.05% tetrahydrozoline. Tetrahydrozoline is a central alpha-1 agonist with a similar effect to clonidine and tizanidine. Effects from unintentional and intentional ingestion may include the narcotic-like effects of sedation, coma, miosis, and respiratory depression along with the cardiovascular effects of bradycardia and hypotension. The toxic dose is poorly defined but may be as small as 2 ml of a 0.05% solution for a child. No fatalities have been reported. The effects from tetrahydrozoline may be more pronounced in children than adults. Overdoses have been reported in both children and adults, but intentional use for chemical submission has not been previously reported. One case report of a suicide attempt in an adult reports the patient learned of the effects of tetrahydrozoline from bartenders and prostitutes that had used it to subdue rowdy customers. Tetrahydrozoline is well absorbed with clinical effects evident in 15 minutes after ingestion.

Tetrahydrozoline will not be detected on routine toxicology investigation. It had been reported to produce false negative results for cannabinoids with urine immunoassay screens.

An underground rumor, with continuance of the rumor by Internet web sites, suggest that putting Visine® in an unsuspecting victim's drink will cause them to have sudden onset of "explosive" diarrhea. It is reported to be a method of "revenge" against difficult customers in bars and restaurants. Gastrointestinal effects from tetrahydrozoline are not supported by the medical literature but the perpetuation of the rumor suggests this may still be used upon unsuspecting victims with unintended and potentially life threatening consequences.

This case suggests that tetrahydrozoline should be added to the list of drugs suspected in cases of chemical submission and sexual assault. Along with other drugs used for this purpose, such as GHB (gamma hydroxybutyrate) and ketamine, tetrahydrozoline may not be detected on routine drug screens.

Tetrahydrozoline, Chemical Submission, Sexual Assault

K21 Arizona Tea, It's Not For Everyone: An Anabasine Accidental Lethal Ingestion

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After attending this presentation, attendees will be afforded a review of poisoning and fatalities due to anabasine, including the symptoms of anabasine toxicity and the procedure for analysis of the compound by GC/NPD and GC/mS.

This presentation will impact the forensic community and/or humanity by providing the forensic community with data from a recent postmortem case in which anabasine toxicity was determined to be the cause of death. There is scant toxicological literature regarding the minimal lethal concentration of this drug, and presentation of this case may help in compiling such data.

The authors will present forensic science information regarding a death attributed to acute anabasine toxicity. Anabasine (3-(2-piperidiyl) pyridine) or neonicotine is the major alkaloid of the plant *Nicotiana glauca*, commonly known as tree tobacco. The shrub can grow up to 6 meters in

height, has large fleshy gray-green leaves, and tubular yellow flowers. The plant has worldwide distribution including Israel, Australia, and South and North America. In Arizona and the desert southwest, it is commonly found along riverbeds. Anabasine ($C_{10}H_{14}N_2$) is similar in chemical structure and pharmacological effects to nicotine. A 50-year-old male transient was living near the river basin of the Salt River outside Phoenix, Arizona. He was witnessed to drink a heated tea-like solution consisting primarily of desert shrubbery and then complained of feeling numb from the level of his mid chest down to his toes. Other transients summoned emergency personnel but resuscitation efforts were unsuccessful, and he was pronounced dead at the scene. The decedent's prior medical history is unknown. A full autopsy was performed approximately 19 hours after death with significant findings being a slightly enlarged heart; moderately congested lungs, and mild diffuse cerebral edema. Routine specimens consisting of femoral blood, urine, vitreous fluid, bile, liver, kidney, brain, and stomach contents were collected for toxicological analysis as well as the tea solution recovered from the scene. What appeared to be leaves were observed in the gastric contents. Blood and urine specimens were subjected to a qualitative analysis using a basic pH drug screen performed by liquid-liquid extraction and analyzed by GC-NPD and GC-MS, with volatiles being assayed by GC-FID. The blood was also screened by ELISA for methamphetamine, benzodiazepines, barbiturates, opiates, and benzoylecgonine, with negative results. A trace amount of methamphetamine was found in the urine by GC-NPD and GC-MS. Quantitative analysis of anabasine was performed on all specimens as follows: briefly, to each tube was added 2 mL of specimen, a 100 uL aliquot of internal standards (0.20 mg/L alpha-phenethylamine, mepivicaine, and dibucaine) and a 100 uL aliquot of concentrated ammonium hydroxide. This was then extracted into 10 mL of n-butyl chloride. A back extraction was performed into 3 mL of 0.2N sulfuric acid. A wash was done with 3 mL of n-butyl chloride and then a 100 uL aliquot of 10N NaOH was added and a re-extraction was done into 10 mL of n-butyl chloride. The solvent was decanted to a conical evaporation tube containing 25 uL of isoamyl acetate and evaporated to 10uL. 1 uL of extract was injected into an Agilent model 6890 gas chromatograph equipped with an Agilent nitrogen-phosphorous detector (NPD) and an Agilent 25 meter HP-5 capillary column (0.33 um film thickness). Split injection (10:1) was done at 260ºC. The temperature program was 60ºC for 1 minute then increased to 315ºC for 5.5 minutes at 9º/minute. Under these conditions the retention time of anabasine was 0.65, relative to mepivicaine. The concentration was determined by comparing the peak area ratios of anabasine to the internal standard against a standard curve with linearity demonstrated up to 1.0 mg/L. Fractional volumes were used for samples exceeding linearity. The concentration of anabasine in the decedent's femoral blood was found to be 0.81 mg/L while tissue levels were: brain 1.11 mg/kg, liver 1.78 mg/kg, kidney 1.58 mg/kg, and gastric 34.4 mg/L. The concentration of the tea like solution was determined to be 151.7 mg/L. Anabasine, like other nicotine alkaloids is rapidly absorbed through the gastrointestinal mucosa as well as by the respiratory mucosa and skin. The symptoms of anabasine poisoning are similar to those of nicotine and include hypersalivation, vomiting, diarrhea, hypertension, tachycardia, diaphoresis, headache, dizziness, twitching, auditory and visual hallucinations, and paralysis. The initial mechanism is stimulation of the nicotine receptors but this may be followed with a blockade at the neuromuscular junction, leading to skeletal and respiratory muscle paralysis. Death is always due to respiratory failure and the few previously reported deaths have occurred within one hour of the onset of symptoms.

Anabasine, Nicotiana Glauca, Gas Chromatography/Mass Spectrometry

K22 How HHS is Applying Recommendations From the Hair Testing Working Group

Carl M. Selavka, PhD, Massachusetts State Police, 59 Horse Pond Road, Sudbury, MA 01776; and Donald J. Kippenberger, PhD, U.S. Army Medical Command (USAMEDCOM), Toxicology Specialty Command, Ft. Sam Houston, TX 78160*

After attending this presentation, attendees will understand how well the recommendations provided by the Hair Testing Working Group to the Department of Health and Human Services have apparently been received and are being incorporated in the current version of the Notice of Proposed Revisions to Mandatory Guidelines for Federal Workplace Drug Testing Programs on alternate matrix testing.

This presentation will impact the forensic community and/or humanity by providing a full list of the areas in which previous HTWG guidance had been incorporated or not followed by HHS in the NPRMG. In addition, since HHS (through SAMHSA and its DTAB process, in all likelihood) will have met several times between the electronic upload of this abstract (deadline of 1 August 2004) and the AAFS meeting (February 2005), feedback will be provided on changes made to the NPRMG in relation to hair drug testing to date.

The Hair Testing Working Group (HTWG) met on four (4) separate occasions from November 1998 to January 2001. These meetings were requested and supported by the Department of Health and Human Services (HHS) Division of Workplace Programs to provide input as the Substance Abuse and Mental Health Services Administration (SAMHSA) began considering and developing rules for active regulatory oversight of alternate matrix drug testing. Over the course of its 4 meetings, the HTWG involved dozens of individuals representing over 10 laboratories, the U.S. Military, ONDCP, RTI and academic researchers in the field.

When the Notice of Proposed Revisions to Mandatory Guidelines for Federal Workplace Drug Testing Programs (NPRMG, FR Doc 04-7984) was ultimately promulgated in April 2004, over 150 public comments were received by HHS. The work of the HTWG had been the subject of significant deliberation and interest by SAMHSA's Drug Testing Advisory Board (DTAB) during the authors' original work from 1998-2001. Therefore, as with many within the laboratory testing industry, MRO population and companies using workplace testing, a great deal of interest in the NPRMG was had. As Co-Chairs of the HTWG, the authors had intimate knowledge of the many hours of discussions from HTWG meetings.

Based on review of the NPRMG, a public comment was forwarded to HHS which spelled out specific areas of the document that were especially outstanding. Also specified are those areas of the NPRMG in which either the field would have reservations or trouble instituting, or for which there appeared to be misstatements about the science involved. For example, it was encouraging that the NPRMG recognized some of the less than favorable elements of urine drug testing approaches and applications and recognized the complementary nature of urine, hair, oral fluid, and sweat drug testing. No single matrix provides the information necessary for every investigation, detection and deterrence strategy, and the NPRMG was clearly building a much better environment for complementary uses of drug testing technologies for the future.

Several of the areas (among many) in which the NPRMG needed changes included: 1) over-discussion in the Preamble of unproven biases that have been suggested among populations of tested individuals; 2) lowering of overall industry standards through the Instrumented Initial Testing Facility (IITF) guidelines outlined in Section M of the NPRMG; 3) clearer guidelines involving metabolites, effective washing techniques and appropriate cutoff levels to differentiate environmental contamination and actual drug ingestion; 4) PT performance standards based more on efficient extraction/recovery procedures; 5) MDMA immunoassay detection recommendations; 6) allowance of body (other than pubic) hair rather than just head hair; 7) minimization of sample handling to prepare duplicates for testing; 8) changes in selected cutoffs and analytes representing drug classes; and 9) unnecessary "invalid sample" collection requirements.

This presentation will provide a full list of the areas in which previous HTWG guidance had been incorporated or not followed by HHS in the NPRMG. In addition, since HHS (through SAMHSA and its DTAB process, in all likelihood) will have met several times between the electronic upload of this abstract (deadline of 1 August 2004) and the AAFS meeting (February 2005), feedback on changes made to the NPRMG in relation to hair drug testing to date will be provided.

Hair Drug Testing, Mandatory Guidelines, HHS/SAMHSA/DTAB Revisions

K23 Bupropion and Its Metabolites in Twenty-Nine Postmortem Cases

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The goal of this presentation is to assist forensic pathologists and toxicologists in evaluation of postmortem concentrations of bupropion and its metabolites.

This presentation will impact the forensic community and/or humanity by assisting forensic pathologists and toxicologists in evaluation of postmortem concentrations of bupropion and its metabolites.

Bupropion is a unique antidepressant unrelated to tricyclic, tetracyclic, selective serotonin re-uptake inhibitors or other antidepressant agents. It is also widely prescribed at low doses for smoking cessation. Bupropion is extensively metabolized via hydroxylation of the tert-butyl side chain to morpholinol-bupropion (M), and via reduction of the carbonyl group to the amino-alcohol isomers, threohydro- bupropion (Threo) and erythrohydro-bupropion (Erythro). Animal studies have demonstrated the morphinol and the amino-alcohol metabolites have approximately 50% and 20% the anti-depressant activity of bupropion, respectively. Postmortem toxicological finding have been reported in only a few overdose cases.

We present the postmortem blood and liver bupropion and bupropion metabolite toxicology findings in 29 deaths: 10 cases of massive ingestion of bupropion where the drug was considered a major contributor to fatal drug overdose; 13 cases of fatal mixed drugs intoxication where there was little indication of excessive bupropion ingestion; and 6 cases of death by natural causes where the decedent was receiving bupropion. Bupropion and its metabolites were isolated from alkalized blood and liver specimens by liquid/liquid extraction with n-butyl chloride/ether mixture. Extracts were back-extracted into acid, extracted with hexane for cleanup and following sample alkalization isolated with butyl chloride. The residues were then analyzed by GC/MS with separation in a DB-5MS column (15m x 0.25mm id x 25 *u*m film thickness) at the following temperatures: initial, 70ºC; ramp, 15ºC/min; finial 250ºC; yielding retention times: bupropion, 7.13; Erythro, 7.87; Threo, 7.98; M, 8.89 and alphaprodine (IS), 8.73 min. Ions monitored for bupropion, Erthyro, and Threo were 44/100/139 m/z; for M, 44/116/224 m/z; and IS, 172/187 m/z. Typical calibrations for all bupropion analytes were from 0.20 - 4.0mg/L. Heart or aorta bupropion and metabolite blood values are given in Table 1 and liver values in Table 2. Femoral blood or other blood specimens from peripheral sites were also analyzed.

Table 1. BLOOD Mean, mg/L (Range, mg/L)

	Bupropion	Erythro	Threo	Morpholinol
Overdose	2.7 (0.28-7.4)	1.4 (0.5-2.8)	11 (2.5-27)	3.1 (1.7-4.2)
Incidental	0.37 (0.1-0.65)	0.43 (0.27-1.1)	2.1 (0.34-5.6)	0.79 (0.57-1.4)
Natural	0.43 (0.26-0.60)	0.51 (0.39-0.75)	2.6 (1.8-4.1)	0.65 (0.51-0.77)

Table 2. LIVER Mean, mg/Kg (Range, mg/Kg)

	Bupropion	Erythro	Threo	Morpholinol
Overdose	5.6 (1.3-16)	6.6 (6.4-15)	81 (40-160)	18 (3.7-61)
Incidental	2.0(0.6-5.4)	2.6 (1.0-4.5)	12 (3.6-50)	4.4 (0.73-17)
Natural	0.59 (0.3-0.7)	2.4 (1.4-4.6)	15 (7.0-17)	2.5 (1.0-5.9)

Only one of the overdose cases was due to bupropion as a single agent. In the other nine bupropion overdose cases, at least one other drug was present in significant toxic amounts; 8 cases involved other antidepressants and 1 involved opiates. Obviously, other drugs were present in the 13 bupropion incidental cases; 9 involved at least one opiate. Comparing blood bupropion data from heart/aorta with peripheral sites revealed no significant postmortem redistribution in these cases. In general, parent bupropion values in blood and liver are good indicators of overdose. Additionally, liver Threo concentrations provide a good discriminater between overdose and therapeutic use. In fatal poisoning, bupropion is seldom encountered as the single causative agent.

Bupropion, Bupropion Metabolites, Fatal Poisoning

K24 Consequences of Introducing a Zero-Concentration Limit for Scheduled Drugs in Blood of Drivers: The Swedish Experience

Alan Wayne Jones, PhD, DSc, Department of Forensic Toxicology, University Hospital, Linkoping, 581 85, Sweden*

The goal of this presentation is to give an overview of driving under the influence of drugs in Sweden before and after a zero-concentration limit was introduced for scheduled drugs in blood of drivers.

This presentation will impact the forensic community and/or humanity by bringing to the attention of the forensic community how a simple change in legislation impacts on traffic law enforcement and the crime of driving under the influence of drugs (DUID).

This presentation gives an overview of driving under the influence of drugs (DUID) in Sweden before and after a zero-concentration limit was introduced for controlled substances in the blood of drivers. The zero-concentration limits apply to illicit as well as prescription drugs if the latter are included on the list of controlled substances. However, medicinal drugs are exempt from the zero-limit law if they were being used in accordance with a physician's prescription. This raises the tricky question of interpreting a measured blood-concentration of a sedative-hypnotic or painkiller and concluding that the person was over-dosing or abusing the substance. This requires careful scrutiny of controlled studies relating C_{max} to the dose and other factors that influence C_{max} e.g., gender, age, adiposity, and disease state. Another confounding factor arises when a drug concentration measured in whole blood, the specimen submitted for forensic toxicology, is compared with concentrations in serum or plasma derived from therapeutic drug monitoring programs. The plasma/whole blood distribution ratios for many drugs of abuse are not well documented.

In connection with the zero-concentration law for controlled substances, the police were allowed to examine the driver's eyes to gather evidence of being under the influence of a psychoactive substance. A small flashlight and pupillometer device were available to measure pupil size and reaction to light and to document any gaze nystagmus that might have existed. In addition, the suspect's behaviour and ability to walk, talk and answer questions were also recorded. Depending on the outcome of these roadside tests, a decision is made to proceed with sampling blood and urine for forensic toxicology.

When urine was submitted for analysis, this specimen was screened for various drug-classes by immunoassay methods (EMIT and CEDIA) and all positive findings were verified by quantitative analysis of blood specimens with GC-MS and GC-NPD detection. The concentration of carboxy-THC and 6-acetyl morphine in urine was determined by LC-MS and GC-MS respectively. Finding a banned substance in blood above the LOQ of the method is sufficient to initiate a prosecution for DUID under the new zero-limit law. The LOQ is different for different substances and might change depending on future developments in the analytical methodology.

The typical DUID suspect in Sweden is a poly-drug user who might combine a stimulant like amphetamine or methamphetamine with a depressant like alcohol or diazepam. Because the punishment for DUID is the same regardless of the number of banned substances identified in blood, this has prompted researchers to re-evaluate analytical routines and in the future plan to verify only a single illicit substance. Since the new law was introduced (July 1999) the number of blood samples submitted by the police for toxicological analysis has increased more than 8-fold. About 90% of specimens contain one or more banned substance. The spectrum of drugs found in blood of drivers has not changed since the new law came into force. Illicit drugs like amphetamine (~50%) and tetrahydrocannabinol (~25%) dominate, followed by diazepam and its metabolite nordiazepam (~15 %), then morphine and codeine (~10%), the metabolites of heroin, and flunitrazepam.

Different countries have their own traditions for dealing with the problem of drug-impaired driving and in European countries the trend is towards zero-concentration limits for illicit drugs. Hitherto, prosecution for DUID required evidence that drugs impaired the person and each suspect was examined by a physician or a drug-recognition expert (mainly in USA). The unequivocal finding of a psychoactive substance in the person's blood and the concentration present provided additional evidence for the prosecution case. Finding an illicit drug in urine was not sufficient to bring a charge of DUID. Because of the development of tolerance and also the short half-life of some drugs, the toxicological results often conflicted with the signs and symptoms reported by the police or the physician. Many prosecutions for DUID were unsuccessful and the police authorities became unwilling to proceed with arresting and charging a person for DUID in borderline cases.

The zero-concentration limit has done nothing to deter people from driving under the influence of drugs. Alcohol and drug abuse are facts of life in modern society and people found guilty of DUID are mostly criminal elements who lack a valid driving permit and have committed other offences. Recidivism is a major problem in DUID suspects in Sweden with over 50% of individuals re-offending within 4 years of their first conviction. How to deal with these traffic delinquents is a major dilemma for the criminal justice system. The zero-limits for controlled scheduled drugs have stimulated police efforts to apprehend offenders and many more successful convictions have been obtained. This has also meant an appreciable increase in the workload for forensic toxicology.

Drugs, Driving, DUID

K25 Purposeful Destruction of a Flat by an Explosion? Forensic Toxicological and Medicolegal Interpretations of an Unusual Case

Werner Bernhard, PhD, Beat Abei, and Martina Gasser, Institute of Legal Medicine, Department of Forensic Chemistry and Toxicology, University of Bern, Buehlstrasse 20, Bern, 3012, Switzerland; and Stephan Bolliger, Department of Legal Medicine, University of Bern, Buehlstrasse 20, Bern, 3012, Switzerland*

The goal of this presentation is to present three recent autopsy cases demonstrating teamwork between pathologist and forensic toxicologist.

This presentation will impact the forensic community and/or humanity by demonstrating optimized teamwork between forensic toxicologists and forensic pathologists to solve an unusual explosion case.

Case circumstances: A thirty-seven-year-old man was found dead under the rubble after an explosion had completely destroyed the flat he inhabited. His three-year-old son, whom he should have returned to his ex-wife several hours prior, and a neighbor were also found dead. The man, who had apparently tried to commit suicide several times in the past, had bought three bottles of camping gas the day before for no apparent reason. The circumstances of the explosion were indicative of a suicide with homicide, the motive probably being the frustration due to the separation from his son. The camping gas bottles had been opened prior to the explosion.

Autopsy: Board certified forensic pathologists performed a conventional forensic autopsy. Toxicology: The body fluids were analyzed with regard to volatiles using a standard method involving two runs on each of two headspace gas chromatographs with flame ionization detectors (2x HS-GC-FID). The screening for drugs and medications was performed by EMIT, GC-MS, and GC-NPD. Confirmation and quantification of Cannabinoids in blood were performed by GC-MS. Results: External examination of the body of the man showed extensive excoriations of the skin of the chest, abdomen, and back. Interestingly, singed hair was detected in the face, namely the beard, eyebrows, and head (with exception of the occipital regions) and the forearms and hands. The autopsy displayed extensive blunt trauma of the chest inner organs and the right arm.

Toxicological analysis of the blood demonstrated a THC level of 6 ng/mL, but no THC-COOH. This is an indication that lighting the joint was the cause of the explosion. The bile, by contrast, showed 27 ng/mL of THC-COOH but no THC. Alcohol could not be detected in the blood. In the blood of the man and the boy, traces of butane and propane gas were detected, indicating they were in the same flat exposed to the gas prior to the explosion. The blood of a neighbor analyzed negative for butane and propane indicating that she was in another flat and not exposed to the gas.

Ignition, Gas Explosion, Cannabis Joint

K26 A Homemade Device to Cheat the Urine Drug Screen

Iouri G. Boiko, MD, PhD, Douglas Posey, MD, Ashraf Mozayani, PharmD, PhD, and Luis A. Sanchez, MD, Harris County Medical Examiner Office, 1885 Old Spanish Trail, Houston, TX 77054*

Attendees will gain awareness of possible cheating in urine drug testing.

This presentation will impact the forensic community and/or humanity by increasing awareness of cheating on drug tests.

The goal of this paper is to report a novel mechanism that may have allowed a male subject to continue the use of illicit drugs while participating in a urine drug-screening program. The mechanism to be described was discovered during a postmortem examination.

Urine drug testing is currently recognized as the "gold standard" for drug testing because of its proven accuracy, reliability, and fairness. It is used to identify users of illicit substances in order to provide security for critical workplaces and to allow monitoring of known drug abusers during treatment. The mechanism described here provides a pathway to "pass" the urine drug screen while continuing the use of illicit drugs.

The recognition and prevention of methods and devices that can be used to alter the urine drug screen process is critical to the success of any drug-testing program. The following are details of the mechanism used in this case to interfere with the screening process.

The decedent was a 21-year-old Caucasian male who was found prone on the bed with his feet touching the floor. The decedent was nude but wearing his glasses. He had numerous tattoos and body piercings that included two penile piercings. There were multiple puncture marks on the body including the antecubital and femoral fossae. The puncture mark on the left upper arm was surrounded by blue-green discoloration. Prescribed medications at the scene included alprazolam, dextroamphetamine, Flonase, ketorolac, OxyContin, and two boxes of Duragesic (fentanyl) patches.

The "novel mechanism" encountered in this case was a container wrapped in duct tape. The container was in a plastic bag, and surgical

tubing ran from the container through the piercing in the penis. According to the decedent's roommate, this device was used to hold urine samples that would be forced through the tubing at the time of urine drug screening. The decedent had a past history of street drug use but had been clean in the recent past. The autopsy findings included multiple old and more recent bilateral injection sites on the antecubital and femoral fossae. The examination of the container and tubing recovered from the scene showed that it could easily be hidden on the body under the clothing, thereby allowing for the replacement of the decedent's urine with a sample known to be free of drugs.

The device described here creates concern about the vulnerability of urine drug screen collection procedures. This case may be the sentinel event that refocuses attention on the conflict between the right of privacy and the need to maintain a safe and secure workplace

Urine Drug Testing, Homemade Device, Urine Substitution

K27 Comprehensive Drugs of Abuse Screening of Overdose Cases By Accurate Mass Liquid Chromatography/ Time-of-Flight Mass Spectrometry

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After attending this presentation, attendees will understand a new analytical technology for identifying drugs of abuse in overdose cases.

This presentation will impact the forensic community and/or humanity by exposing the attendees to a new approach to identification and quantification of drugs of abuse that can be applied to poisons and other toxicological investigations.

Comprehensive drug screening of overdose patients is presently performed by GC/MS. The methodology includes extraction and analysis of both acidic and basic drugs. Over 70 drugs of abuse are included in the standard screen. The methodology is both laborious and time consuming and includes derivatization steps for many of the targeted compounds. In addition, there are cases where the screen does not produce results indicative of the overdose. The speed of analysis can be a critical issue where the patient/victim is unconscious and proper medical attention may depend on identification of the unknown toxin. Even in these cases where toxicological signs indicate a drug overdose, the analytical methodology may be slow.

This work will show the comparison of present analytical methods using GC/MS with Liquid Chromatography/Time-of-Flight Mass Spectrometry (LC/TOF MS). Acid and base extraction of blood serum and their analysis using an Agilent LC/MSD TOF with reversed-phase chromatography is used for fast drug screens of overdose patients. This instrumentation has been shown to provide routine mass accuracy measurements better than 3 ppm for compounds with mass above 200 amu. This technology combined with the ability to perform fast reversed-phase chromatography is used to develop a drug screen without the need for derivatization. The screen will examine the more than 70 compounds targeted by GC/MS and include designer drugs and other drugs of abuse not presently sought. The results will be evaluated for quantitative accuracy and precision, qualitative confidence, and overall speed of the analysis. In addition, the ability to use the accurate mass measurement capability of the technology to propose an identification for peaks found in the screen that are not among the comprehensive list of target compounds will be determined and presented. The results will be summarized so that feasibility of this new technology can be assessed.

Time-of-Flight, Mass Spectrometry, Drugs of Abuse

K28 Fluorescent Derivatization for Trace Detection of Opiates and Other Drugs of Abuse by Capillary Electrophoresis

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Attendees will learn a number of new procedures for the fluorescent derivatization and detection of tertiary amines such as opiates and other drugs of abuse using capillary electrophoresis with laser induced fluorescence.

This presentation will impact the forensic community and/or humanity by providing improved methods for the trace detection of drugs of abuse by capillary electrophoresis.

The described procedure involves a facile demethylation followed by a fluorescent derivatization reaction that can be used by forensic practitioners to determine 6-monoacetyl morphine and other tertiary amines at ultra trace levels. Highly selective conditions are then described for the separation and detection of these compounds using capillary electrophoresis with laser induced fluorescence. In addition, other fluorescent derivatization reactions are utilized for the analysis of primary and secondary amines such as benzyl piperazine using diode lasers with capillary electrophoresis.

Capillary electrophoresis (CE) methods are becoming increasingly popular as screening tools for forensic drug analysis. However, most separations using CE involve UV detection with relatively short detection window pathlengths when compared to HPLC. This limits sensitivity. While a number of useful techniques have been developed for sample preconcentration based on field amplified sample stacking, (especially for basic drugs) there still is a need for improved detection for toxicological samples. One of the best and most successful ways to improve CE detection limits is with laser-induced fluorescence. Because native fluorescence is limited to only a few compounds, most drugs of abuse need to be derivatized. This derivative should be fairly polar for best compatibility with CE. Unfortunately most fluorescent derivatization reactions involve reactive dyes that interact mainly with primary and secondary amines. Compounds such as opiates and cocaine that contain tertiary amines will not react with these dyes. In this project researchers explore methods for generation of secondary amines from these compounds and examine a variety of derivatization reactions for compatibility with capillary electrophoresis separation methods.

Spiked urine samples were extracted using Bond Elute Certify SPE (Varian) columns following manufacturer's suggested protocols. Samples were then diluted in dichloroethane and reacted with 50 microliters of 1-chloroethyl chloroformate by heating to reflux for 2-4 hours. The solvent was then removed, and the sample was pH adjusted to 8.5 with bicarbonate and reacted for 30 minutes with fluorescein isothiocyanate. The resulting compounds produced a fluorescent emission at wavelengths above 520 nm that was compatible with commonly used 488nm argon-ion lasers. Alternatively, samples were reacted with the dye Cy5 NHS ester in a mixture of triethyl amine and DMSO. These samples were analyzed using an inexpensive diode laser operating at 635nm with emission at 665nm.

Separation of derivatized samples such as opiates, which have very similar structures, can be particularly challenging by any method. To perform these separations by capillary electrophoresis, beta-cyclodextrins were added to the buffer in order to form highly specific inclusion complexes with the derivatized drugs. In addition, altering the formation constants of these complexes using a mixture of organic solvents further optimized separations. Sample analysis was performed using a Beckman P/ACE MDQ capillary electrophoresis system with LIF detector, and the method was developed to be compatible with microfluidic devices. The results provided a highly sensitive screening tool for specific drugs of abuse with detection limits as low as 50pg/mL.

Capillary Electrophoresis, Drug Analysis, Laser Induced Fluorescence

K29 Motor Vehicle Passive Cannabis Smoke Exposure and Intercept® Oral Fluid Testing

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The goal of this study was to determine if extreme passive exposure to cannabis smoke in a motor vehicle would produce positive results for delta-9-tetrahydrocannabinol (THC) in oral fluid tests.

"Passive cannabis smoke exposure" is an explanation offered by cannabis users for positive urine tests conducted in workplace programs. This defence has not been reported in more than 15,000 oral fluid positive cannabis tests in workplace programs, but might be attempted. This presentation will impact the forensic community and/or humanity by providing results from this study that demonstrate that such claims have no scientific basis absent the extreme conditions described. This information is essential in interpretation of oral fluid tests by forensic toxicologists and Medical Review Officers.

The objective of this study was to determine if extreme passive exposure to cannabis smoke in a motor vehicle would produce positive results for delta-9-tetrahydrocannabinol (THC) in oral fluid tests.

Passive exposure to cannabis smoke in an unventilated room has been shown to produce a transient appearance of THC in oral fluid for up to 30 minutes (1,2). However, it is well known that such factors as room size, ventilation conditions, and extent of smoke exposure can affect outcome results.

The authors conducted a passive cannabis study under extremely severe passive smoke exposure conditions in an eight-passenger van. The van had an approximate interior volume of 15.3 cubic meters. Four experienced, male cannabis users each smoked a single cannabis cigarette (mean 5.4 %THC) while seated inside the closed van in the presence of four passive, drug-free, male non-smokers. There were four rows of seats in the van; one cannabis smoker sat on each row alongside one passive subject. Cannabis cigarettes were lighted by the cannabis smokers in the van and smoked for approximately 20 minutes to completion. All doors and windows were closed and the van was turned off, providing no ventilation. After the completion of cannabis smoking, all participants remained in the closed, unventilated van for an additional 60 minutes.

Oral fluid specimens were collected with the Intercept® Oral Specimen Collection Device (OraSure Technologies, Bethlehem, PA) according to manufacturer's instructions. Oral fluid collections were made inside the van for the first 45 minutes. Participants were allowed outside the van after 60 minutes where specimen collection continued. Bilateral oral fluid collections (left and right side of the mouth) were made from all subjects at the following times: baseline; 0 (immediately at the end of smoking); 15, 30, 45 minutes inside the van, and 1; 1.25; 1.5; 1.75; 2; 2.5; 3; 3.5; 4; 6; and 8 hours outside of the van, and from passive subjects only at 10; 12; 24; 36; 48; 60; and 72 hours.

Oral fluid specimens were analyzed with the Cannabinoids Intercept® MICRO-PLATE Enzyme Immunoassay by OraSure Technologies (Bethlehem, PA) following manufacturer's procedures. Quantitative analysis of THC in oral fluid specimens was performed by GC-MS-MS. THC concentrations were adjusted for dilution (X3) and are reported as estimated neat oral fluid concentration. The screening and confirmation cut-off concentrations for THC in neat oral fluid were 3 ng/mL and 1.5 ng/mL, respectively. The LOD/LOQs for THC in the GC-MS-MS assay were 0.3/0.75 ng/mL.

Screening and GC-MS-MS results for the bilateral (simultaneous) oral fluid collections are shown side-by-side in Table I. Only results for specimens that tested positive in screening or GC-MS-MS were tabulated. The remaining oral fluid specimens collected from one through 72 hours tested negative in screening and confirmation with the exception of one specimen that appeared to be contaminated during handling of the Intercept collection device. The apparent contaminated specimen, collected at 2.5 hours by PASSIVE #C, screened positive and confirmed with a THC concentration of 3.0 ng/mL. The accompanying bilateral specimen collected simultaneously with the contaminated specimen screened negative and was negative for THC by GC-MS-MS at LOD.

Table I. THC Oral Fluid Screening (cutoff = 3 ng/mL) and Confirmation (cutoff = 1.5 ng/mL) Results for Passively Exposed Subjects (two specimens per time point, collected bilaterally).

	PASSIVE #A		PASSIVE #B		PASSIVE #C		PASSIVE #D		Mean
Minutes	THC Screen	GC-MS-MS ng/mL	THC Screen	GC-MS-MS ng/mL	THC Screen	GC-MS-MS ng/mL	THC Screen	GC-MS-MS ng/mL	GC-MS-MS (SEM), ng/mL
0	+/+	4.8/3.6	+/+	6.0/7.5	+/+	6.6/5.1	+/+	3.9/4.5	5.3/5.2 (0.6/0.8)
15	+/+	4.2/6.0	-/-	2.7/2.8	-/-	<1.5/1.8	+/+	3.9/2.3	3.6/3.2 (0.4/0.9)
30	-/+	3.3/4.8	-/-	2.4/1.6	+/-	3.0/<1.5	+/-	2.8/2.9	2.9/3.1 (0.2/0.8)
45	-/-	2.0/1.7	-/-	<1.5/<1.5	-/-	<1.5/<1.5	-/-	<1.5/2.6	2.0/2.1 (NA/0.3)

This study confirms and extends earlier findings (1,2) on the effects of passive exposure to cannabis smoke on oral fluid results. The risk of a positive test result in screening and confirmation for THC was limited to 30 minutes or less following passive cannabis smoke exposure under extreme environmental conditions.

The extreme nature of the conditions employed in this passive cannabis smoke study is worthy of comment. Each passively exposed subject remained seated alongside a cannabis smoker during the hour of passive smoke exposure inside the van. The cannabis smokers smoked cannabis cigarettes to completion. The van doors and windows remained closed throughout the study and the van was turned off, providing no ventilation. Oral fluid collections were made for the first 45 minutes inside the van in the presence of cannabis smoke further increasing the risk of environmental contamination during collection. Given the extreme nature of the conditions employed in this study, it is concluded that the risk of positive oral fluid tests from passive cannabis smoke exposure would not occur under realistic conditions.

References:

1. R.S. Niedbala, K.W. Kardos, S. Salamone, D.F. Fritch, M. Bronsgeest and E.J. Cone. Passive cannabis smoke exposure and oral fluid testing. *J. Anal. Toxicol.* In press (2004).
2. R.S. Niedbala, K.W. Kardos, D.F. Fritch, S. Kardos, T. Fries, J. Waga, J. Robb, and E.J. Cone. Detection of marijuana use by oral fluid and urine analysis following single-dose administration of smoked and oral marijuana. *J. Anal. Toxicol.* 25: 289-303 (2001).

Cannabis, Passive Exposure, Oral Fluid

K30 An Unusual Case of Homicide by Chronic Methanol Poisoning

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The goal of this presentation is to present the forensic community with an unusual agent and method of homicide due to chronic methanol poisoning.

This presentation will impact the forensic community and/or humanity by alerting toxicologists that methanol can be used as an agent of

murder and is often initially misdiagnosed. In addition, it will alert the community that beverages can be used as a route to administer poisons chronically.

An unusual case of homicide by chronic methanol poisoning is presented. Prior to his poisoning, the victim a 37 yr-old man, was in good health and physically active, exercising and playing sports. Approximately, one month prior to his death, he complained of intermittent gastric distress, nausea, and episodes of shortness of breath. When his symptoms first developed, his physician considered heart disease; however, he underwent a stress echocardiogram that yielded normal findings. After a family gathering, the victim awoke the next morning "feeling sick." Despite burning in his throat, nausea, and shortness of breath he went to work; however, his symptoms increased during the day and he returned home. His gastric distress worsened, he vomited ten times, and his breathing became labored, at which point EMT's were called and he was transported to the hospital. On admission, he complained of severe gastrointestinal pain and tenderness, he was diaphoretic, tachycardic, mentally confused, and tachyapneic with labored breathing. Initial chemistries revealed a severe metabolic acidosis; pH 7.07; HCO_3^-, 2.3 meg/L; pCO_2, 8.0 mm Hg; glucose, 181 mg/dL. His calculated ion gap was 28 and osmol gap was 28. Serum toxicology findings were: methanol, 750 mg/L; other volatiles including ethanol, negative; ethylene glycol, negative; salicylate 2.8 mg/dL; acetaminophen and tricyclic antidepressants, negative. Despite hemoperfusion and ethanol antidote administration, the patient developed multi-organ failure and was pronounced dead two days after admission. The investigation revealed that the victim had no occupational or recreational exposure to methanol. The victim had a history of ingesting the nutritional supplement creatine. His wife would mix a large tablespoonful of this powder into 20 fluid ounce bottles of Gatorade. The victim had ingested such a bottle of Gatorade the evening before his hospitalization. Police recovered a bottle of prepared creatine/Gatorade from the victim's home refrigerator, two more bottles from a refrigerator at the victim's workplace and a 1/3 full bottle on his desk at work. All these items were found to contain approximately 1 fluid ounce of pure methanol. The recent medical history of the victim and toxicology findings were consistent with chronic exposure to methanol with increasing or an increased dose resulting in a fatal accumulation of the toxic metabolite formic acid. His continued physiological deterioration prior to and during his hospitalization, despite heroic treatment, is consistent with the delayed severe toxicity of methanol. The family member was convicted on charges of "first degree murder."

Homicide, Methanol, Poisoning

K31 Internet-Advertised Drug-Removal Products: Effects on Cocaine, Opiates, and Carboxy-THC in Hair

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The goal of this presentation is to show that products commonly advertised to remove drugs from hair only remove drugs from the surface of the hair samples. Various screening and extraction methods will be discussed.

This presentation will impact the forensic community and/or humanity by clarifying the methodologies required for reliable hair analysis.

Numerous products are advertised on Internet websites as being effective at removing drugs from hair. To test the effects of these products on results of analysis of hair for the presence of cocaine, opiates, and carboxy-THC, hair from a user of these drugs was treated with eight different products according to package instructions of the respective products. As a control, the hair was also treated with Prell shampoo. Following the Prell and experimental treatments, the hair was washed and analyzed for the presence of the drugs by routine procedures. Products tested included Bio-Cleanse™, Dr. Potter's Detoxifying Hair Mudd, All Drugs Follicle Cleanse, Totally Clean, Clear Choice, testPure All-In-One Cleansing Shampoo & Conditioner, AllClear Hair Purifying & Cleansing System. In addition, Nexxus Aloe Rid Shampoo and Clarifying Treatment were tested because, although they are not Internet products, there are websites claiming that use of these can remove drugs from hair. After Prell treatments, the washed hair contained an average of 152 (+ 27, S.D.) ng cocaine/10 mg hair, 12.5 (± 2.5 ng, S.D.) morphine/10 mg hair, and 3.9 (average of duplicates) pg carboxy-THC/10 mg hair. After application of the various "removal" products, the results were essentially the same as the Prell results: 108 –177 ng cocaine/10 mg hair; 8.3 – 14.8 ng morphine/10 mg hair; and 2.6 – 4.4 pg carboxy-THC/10 mg hair. These products were thus shown to be ineffective, essentially equivalent to normal shampooing, at removing that drug in hair that is resistant to removal by effective laboratory washing.

However, for methods that do not extract the hair sample's full drug content for analysis, the products would require testing under the actual extraction conditions. It is known from a study performed in Psychemedics laboratory of cocaine users, for example, that contamination of users' hair samples with cocaine ranged from almost none to 20 times the amount of the hair content after washing. Therefore, screening methods that only partially extract the drug from the hair prior to analysis may not detect a positive sample that has been well cleansed of surface contamination by a cosmetic product. In such cases, the effects of various hair care and drug removal products should be tested under the conditions in use to detect positives.

Hair Analysis, Cocaine Opiates C-THC, Cosmetics Treatments

K32 Forensic Entomotoxicology: A Study in the Deposition of Amphetamines Into the Larvae of the Black Blow Fly, *Phormia regina*

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The goal of this presentation is to better understand the potential implications of using insects as a toxicological specimen.

This presentation will impact the forensic community and/or humanity by helping to build a database to better understand the utility using insects as a toxicological specimen.

Due to events in severe decomposition, either no soft tissue remains on which to perform a toxicological analysis or putrefactive fluids complicate and interfere with the analysis of the soft tissues. The purpose of this experiment was to study the trends in the deposition of amphetamines into the larvae of the black blow fly, *Phormia regina*, in order to better understand the value of entomological evidence as toxicological specimens. Drug deposition was analyzed by linear regression to find a correlation between whole larvae drug concentration and food source drug concentration. *P. regina* larvae were raised at 21ºC on pork homogenized with three concentrations of ephedrine (36.5, 73, 146 mg/kg), methamphetamine (1.5, 3, 6 mg/kg), and fenfluramine (25, 50, 100 mg/kg). The middle dosage of each drug reflected the LD_{50} of the drug in rabbits. At the end of the feeding stage, the larvae were harvested, washed, and frozen. Ten larvae were subsequently homogenized, diluted in 2 ml water, and subjected to a liquid-liquid extraction. The extracted drugs were derivatized with HFBA and analyzed by GC/MS. In the concentration ranges investigated, the concentrations of ephedrine, methamphetamine, and fenfluramine found in the larvae correlated with the concentration of the drug in the food source (R^2 = 0.9081, 0.9886, and 0.8302 respectively). Fenfluramine was the only drug which biotransformed in the larvae to a

known metabolite, norfenfluramine. The concentrations of fenfluramine and norfenfluramine were added to reflect the total concentration of "fenfluramine" extracted from the larvae. As a result, the concentration of total fenfluramine in the larvae was found to correlate more strongly (R^2 = 0.9107) with the concentration of drug in the food source. The data showed that with increasing concentrations of drug in the food source, the more drug that was accumulated in the larvae and subsequently extracted.

Entomology, Toxicology, Amphetamines

K33 The Quantitation of Sildenafil (Viagra®) and its Metabolite (UK-103,320) in Postmortem Specimens Using LC/MS/MS/MS

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Attendees will be briefed on an analytical method for the quantitation of sildenafil and its active metabolite in postmortem fluid and tissue specimens.

This presentation will impact the forensic community and/or humanity by demonstrating an introduction to applicable LC/MS methodology.

During the investigation of fatal civil aviation accidents, postmortem samples from accident victims are submitted to the FAA's Civil Aerospace Medical Institute for toxicological analysis. The FAA Laboratory develops analytical methods for the identification and quantitation of compounds that may be encountered. This presentation describes a rapid and reliable method for the identification and quantitation of sildenafil (Viagra®) and its active metabolite, UK-103, 320, from postmortem tissues and fluids. This procedure incorporates solid-phase extraction and LC/MS/MS/MS utilizing an atmospheric pressure chemical ionization (APCI) ion trap mass spectrometer (MS) in the positive ionization (PCI) mode. Solid-phase extraction provided an efficient sample extraction yielding recoveries of approximately 80%. This method is highly selective and sensitive, having a limit of detection of 1 ng/mL for both compounds. Sildenafil and UK-103, 320 were found to have a linear dynamic range of 2-800 ng/mL and 4-800 ng/mL, respectively. This procedure showed intra-day (within day) relative errors of £ 6% and relative standard deviations (RSDs) within 4% for both 50 ng/mL and 200 ng/mL controls. The inter-day (between day) relative errors were £ 4%, while the RSD was within 12% for both control concentrations. Sildenafil and UK-103,320 were shown to be stable in blood for at least one week at 4°C. This method was applied to fluid and tissue specimens collected from two separate fatal aviation accident victims. The concentrations of these two compounds in various specimens will be discussed.

Sildenafil, LC/MS, Postmortem

K34 Methylenedioxymethamphetamine (MDMA)-Related Deaths in Ontario, Canada (1999-2002)

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Attendees will be provided a retrospective review of the role of MDMA (3, 4 methylenedioxymethamphetamine) in sudden unexpected deaths in the province of Ontario, Canada over the four-year period from 1999 to 2002.

This presentation will impact the forensic community and/or humanity by adding to the current database of knowledge regarding postmortem blood MDMA concentrations. Exemplar cases to be included in the oral presentation will also further understanding of the type of toxicities observed following MDMA overdose. One of the unique aspects of this research is that it provides information for a region (Ontario, Canada) that has not yet been documented in the scientific literature. However, this research shows that the data for Ontario concurs with previously published research from the U.S. and abroad.

Introduction: MDMA and its pharmacologically active metabolite methylenedioxyamphetamine (MDA) were initially synthesized for use in clinical practice as appetite suppressants but have evolved as popular street drugs. In particular, MDMA ("Ecstasy," "Love Drug," "E") has been associated with the dance music community and all-night rave parties as a result of its CNS stimulant effects, which allow users to resist fatigue; and its mild hallucinogenic properties that enhance the visual light shows at these venues. As a result of the current popularity of MDMA, it is often the role of the forensic toxicologist to interpret blood concentrations of this drug. It has been noted, however, that there are difficulties in interpreting postmortem concentrations of MDMA and MDA. Fatal MDMA concentrations have been shown to vary widely depending on the circumstances under which the drug is administered. This data will further the understanding of MDMA blood concentrations through (1) comparison of MDMA blood concentrations in deaths attributed solely to MDMA intoxication with blood concentrations in cases where MDMA was deemed incidental, (2) exemplar case histories, and characterization of the circumstances surrounding MDMA-related deaths and (3) review of the demographic characteristics of MDMA-related deaths in the current study and in the scientific literature.

Methods: MDMA-related deaths were retrospectively identified from the files of the toxicology sections of the Centre of Forensic Sciences and the Northern Regional Laboratory, which provide the sole toxicology testing for coroner's investigations in the province of Ontario (approx. population 12 million). Inclusion criteria were the: time periods between 1999 and 2002 and the detection of MDMA and/or MDA in postmortem blood. Further case history information pertaining to the circumstances of death, autopsy findings, and cause and manner of death was obtained from the Office of the Chief Coroner of Ontario.

Identification of MDMA and its major metabolite MDA (methylenedioxyamphetamine) were by GC-NPD and GC/MS following liquid-liquid extraction. Quantitation of MDMA and/or MDA was by GC-NPD after derivatization with acetic anhydride.

Results & Discussion: MDMA and/or MDA were detected in 37 postmortem cases in the province of Ontario for the years 1999 to 2002, inclusive. The typical MDMA-related death was young (mean age=26 years) and male (n=33). Although the range of ages observed was 16 to 50 years, 74% of individuals were found to be less than 30 years of age at the time of their death.

The mean blood MDMA concentration in deaths attributed solely to MDMA intoxication was 6.3 mg/L (n=11, range=0.4-27 mg/L) with corresponding MDA concentrations ranging from traces (<0.1 mg/L) to 3.8 mg/L. This concentration range was found to overlap with MDMA blood concentrations detected in traumatic deaths (e.g. GSW, MVA, drowning) where MDMA was considered an incidental finding (n=14, mean=0.7 mg/L, range=traces-2.5 mg/L). MDA concentrations ranged from undetectable to 0.1 mg/L. The remaining 12 MDMA-related deaths were attributed to overdose with a drug other than MDMA (e.g. heroin (n=3), methadone (n=1)) or were ruled to be mixed-drug intoxications by the investigating coroner. Blood MDMA concentrations in these cases were similar to concentrations observed in the traumatic deaths (mean=0.7 mg/L, range=undetectable-1.9 mg/L). MDA concentrations were also similar, with the exception of one, mixed-drug intoxication case, which was found to have a blood MDA concentration of 12 mg/L.

On an annual basis, the number of MDMA-related deaths over the time period studied did not change. However, a trend in the circumstances under which MDMA was taken was noted. For example, fatal MDMA intoxications in more recent years were less likely to be associated with rave parties (0/3 deaths in 2002). This observation may be due to the influence of harm reduction organizations as well as a decreasing trend towards all-night rave parties.

MDMA, Drug Concentrations, Postmortem

K35 Interpretation of Glucose and Lactate Levels in Postmortem Vitreous Fluid

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After attending this presentation, attendees will attain a more in-depth understanding of the interpretation of postmortem levels of glucose, lactate, and electrolytes.

This presentation will impact the forensic community and/or humanity by making the forensic scientist understand the advantages and the shortcomings with vitreous fluid analysis, and how hyperglycemia may be diagnosed postmortem.

Background: In order to identify an antemortem hyperglycemia in postmortem cases, it has been suggested that d-glucose levels could be used. Since one glucose molecule during anaerobic conditions is converted to two lactate molecules several investigators have proposed that the sum of the glucose and the double of lactate levels be used in postmortem cases. The authors decided to study the vitreous concentrations of glucose, lactate and potassium in a large number of cases to evaluate the use of this data in medicolegal investigations.

Material and methods: 0.15 mL vitreous fluid was gently aspirated from the center of the eye (Cloquets canal) from 374 consecutive cases as soon as possible after arrival of the body to the morgue. The fluid from both eyes was pooled. Glucose, lactate and electrolytes were analyzed with ion-specific electrodes, using a Radiometer AVL500 blood gas instrument. A separate study was conducted on whole-vitreous samples; the samples were vortex-mixed, and half of the fluid was then transferred to a separate tube containing NaF at a concentration of approx 1%. Both samples were centrifuged and analysis was carried out on the supernatant and the pellet. In addition, separate samples were treated similarly, but were also subjected to sonication before centrifugation.

Results: Mean postmortem glucose levels in the consecutive cases was 0.99 mmol/L, but the median was as low as 0.1 mmol/L. Lactate levels increased linearly with time after death, as assessed by the vitreous potassium concentration, except for a minor proportion of cases that still showed low lactate levels even after long postmortem intervals. There was no obvious drop in glucose levels with increasing potassium levels, suggesting that glucose stays stable after the initial phase. In three cases, very high glucose levels were found (57, 46, and 23 mmol/L), and the cause of death was certified as hyperglycemia. In additional cases hyperglycemia might have contributed to death. Regarding "whole-vitreous" samples, the addition or omission of NaF did not affect the concentrations of glucose, lactate, or potassium. Further, analysis of the supernatant and the pellet after centrifugation yielded the same results. Sonication of the samples before centrifugation did not affect the results either. Re-analysis of several samples after long periods of storage showed similar results as the primary analysis.

Conclusion: Vitreous fluid is a robust matrix, and suitable for postmortem chemistry. As to the analysis of electrolytes, glucose and lactate, it is not necessary to centrifuge the samples, or to add fluoride, to avoid further changes. To estimate the antemortemantemortem blood glucose levels, d-glucose alone should be used. Lactate is of no value for the diagnosis of hyperglycemia.

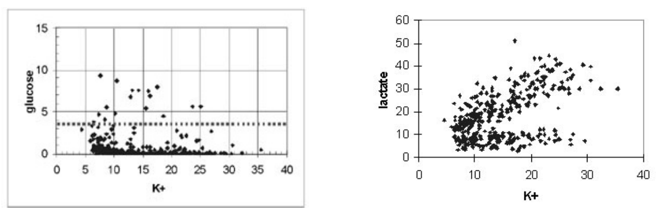

Fig 1. (left) Elevated glucose levels may be found even at long postmortem intervals (highest values not shown). (right) Lactate values do not assist in the diagnosis of hyperglycemia.

Postmortem Chemistry, Glucose, Fatal

K36 Investigation of Cocaine Metabolite Concentrations in Postmortem Cases

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Attendees will learn the cocaine metabolic pathway; understand the methodologies used for quantitatively measuring cocaine and 13 cocaine metabolites in postmortem blood and urine; and acquire information concerning cocaine metabolite concentrations in postmortem specimens.

This presentation will impact the forensic community and/or humanity by providing preliminary data to suggest that analysis of minor cocaine metabolites may aid in the differentiation of cocaine from non-cocaine related deaths.

Toxicological testing of apparent cocaine-related deaths typically involves identification, confirmation, and quantification of the parent analyte (cocaine) and a selection of cocaine metabolites, for example: cocaine (COC), benzoylecgonine (BE), cocaethylene (CE), and ecgonine methyl ester (EME). CE is produced if cocaine is used with ethanol. Other analytes that may be measured include anhydroecgonine methyl ester (AEME), a pyrolysis product, and norcocaine (NCOC). A previous study of 13 cases [Jenkins and Goldberger, *J. Forensic Sci.* 42(5): 824-827, (1997)] found no relationship between cause of death and concentrations of cocaine, BE, EME, and/or CE. However, it is not known if these analytes in addition to other cocaine metabolite concentrations could be useful in understanding cocaine related deaths.

This study examined a total of 13 cocaine metabolites from 103 cases, containing both cocaine and non-cocaine related deaths in postmortem blood and urine. The analytes of interest included AEME, EME, ecgonine ethyl ester (EEE), NCOC, norcocaethylene (NCE), *o,m,p*-hydroxycocaine (*o,m,p*-HOCOC), CE, norbenzoylecgonine (BNE), and *o,m,p*-hydroxybenzoylecgonine (*o,m,p*-HOBE). The COC and BE findings for 100 of these cases have been previously reported [Jenkins, Levine, Titus, and Smialek, Forensic Sci. Int. 101:17-25 (1999)] and will not be discussed in this report.

Heart blood and urine specimens from postmortem cases were analyzed according to a previously published method [Cone, Hillsgrove and Darwin, Clin. Chem. 40 (7): 1299-1305 (1994)]. Briefly, buffered specimens were extracted with calibrators and controls using deuterated internal standards by solid phase extraction followed by gas chromatographic/ mass spectrometric analysis of the silyl derivatives.

Cases were divided into 2 groups for evaluation: those cases in which "cocaine intoxication" was listed as the cause of death were classified as cocaine related; and those for which cocaine intoxication was not listed in the cause of death were classified as non-cocaine related deaths. This latter group included gunshot wounds, drowning, asphyxia, blunt force injuries, as well as deaths determined to be due to other drugs (narcotics, alcohol, N=15).

There were 34 cocaine related deaths. Metabolites were grouped according to the prevalence in which they were found positive in the various cases. Other than BE (previously reported) the two most common metabolites detected were EME (N=33 for blood, N=34 for urine), followed by *m*-HOBE (N=29 for blood, N=34 for urine). The concentration ranges (mean +/- SD) of EME in blood and urine specimens were 16-6413 ng/ml (835.8 +/- 14.9 ng/ml) and 6-179524 ng/ml (11183.7 +/- 309.3 ng/ml), respectively and for *m*-HOBE, the concentration ranges were 4-563 ng/ml (72.9 +/- 14.7) and 5-166804 ng/ml (5190.4 +/- 281.7), respectively. In the blood specimens, other analytes were found in the following order, from most common to least common: *p*-HOBE (N=29); EEE, CE, and *o*-HOBE (N=25); *p*-HOCOC (N=21); *m*-HOCOC (N=18); AEME (N=14); BNE (N=13); NCOC (N=12); *o*-HOCOC (N=9); NCE (N=2). However, the urine specimens demonstrated a slightly different prevalence: NCOC (N=33); *m*-HOCOC, CE, *p*-HOBE (N=32); EEE (N=31); *p*-HOCOC (N=29); BNE (N=28); NCE (N=27); *o*-HOBE (N=15); *o*-HOCOC (N=9).

There were 69 non-cocaine related deaths. In these cases, apart from BE, the same two common metabolites in cocaine-related deaths were most prevalent: EME (N=68 for blood, N=69 for urine) and *m*-HOBE (N=52 for blood, N=69 for urine). The concentration ranges (mean +/- SD) of EME in blood and urine were 2-717 ng/ml (155.6 +/- 16.4) and 28-54939 ng/ml (6112.9 +/- 108.7) respectively and for *m*-HOBE, the ranges and mean concentrations were 1-1171 ng/ml (43.7 +/- 15.0) and 7-62751 ng/ml (3284.4 +/- 110.8) respectively. In the blood specimens, other analytes appeared in the following order, from most common to least common: *p*-HOBE (N=49); CE (N=46); *o*-HOBE (N=43); *p*-HOCOC (N=40); EEE (N=33); *m*-HOCOC (N=32); AEME (N=15); BNE (N=10); *o*-HOCOC (N=8); NCOC (N=7); NCE (N=6). For the urine specimens prevalence was as follows: NCOC (N=69); *m*-HOCOC, *p*-HOBE (N=68); CE (N=67); EEE (N=65); *p*-HOCOC, BNE (N=58); NCE (N=53); AEME (N=51); *o*-HOBE (N=39); *o*-HOCOC (N=24).

Minor metabolites of cocaine are readily detectable in postmortem specimens. It appears the most prevalent minor metabolites detected in both cocaine and non-cocaine related deaths were *m*-HOBE and *p*-HOBE. However, there were some differences between the two groups. In blood, AEME, EEE, *o*-OHCOC, and BNE were more than twice as likely to be present in cocaine related deaths and NCOC was more than three times as likely to be present than in non-cocaine deaths. More variability was observed with the urine data. The data demonstrated that the mean concentrations of the majority of metabolites in blood and urine were lower in the non-cocaine deaths than the cocaine-related deaths, except for NCE and EEE. This study has provided preliminary data to suggest that analysis of minor cocaine metabolites may aid in the differentiation of cocaine from non-cocaine related deaths.

Forensic Science, Toxicology, Cocaine Metabolites

K37 Topiramate (Topamax®) Positive Death Investigation and Impaired Driving Cases in Washington State

Ann Marie Gordon, MA, and Barry K. Logan, PhD, Washington State Toxicology Laboratory, 2203 Airport Way South, Suite 360, Seattle, WA 98134*

After attending this presentation, attendees will understand that topiramate is increasingly prescribed for seizure disorders and off-label use. This presentation gives context for evaluation of topiramate blood concentrations in two populations, death investigation cases, and suspected impaired drivers.

This presentation will impact the forensic community and/or humanity by providing information as to the topiramate concentrations detected in two populations, death investigations and suspected impaired drivers, and will assist other forensic toxicologists in interpreting the level of this drug in their own cases.

Topiramate (Topamax®) has been available since 1996 and has proven very effective for treating seizure disorders. As with many other anti-epileptic drugs (AED), topiramate has recently gained attention for its off-label use. A search in PubMed® disclosed articles describing topiramate use for the treatment and prevention of migraines, cluster headaches and childhood headaches; psychosis, mania, schizophrenia, bipolar disorder, depression and kleptomania; eating disorders including bulimia, binge eating obesity, anorexia nervosa, and as adjunct therapy to treat weight gain with olanzapine, SSRIs and other anti-psychotic medications; neuropathic pain; alcohol dependency and craving, morphine dependency; and benzodiazepine withdrawal. One article even described its use in treatment of refractive scars.

The effects of topiramate are concentration dependent and according to the manufacturer, not subject to the development of tolerance. Dosage for anti-seizure therapy ranges from 200 to 800 mg/day. Side effects include sedation, dizziness, ataxia, speech difficulty, nystagmus, and paresthesia. Metabolic acidosis has been reported in 2 cases.

Peak plasma concentrations in patients stabilized on 800 mg/day have been reported at 5.5 mg/L. Blood/plasma ratios are inversely proportional to concentration averaging 7.1 at a blood concentration of 3 mg/L and 1.3 at a blood concentration of 15 mg/L.[1] Mozayani *et al.*[2] reported a topiramate overdose with blood levels of 8.9 mg/L, and Langman *et al.*[3] reported a fatal topiramate toxicity with a postmortem central blood concentration of 170 mg/L.

In an effort to evaluate the role of topiramate in human performance and death investigation casework, the authors reviewed the findings in all positive topiramate cases from 1998 to June 2004.

Topiramate was first detected in a death investigation case in 1998. Since then the authors have reported 107 cases positive for topiramate; 51 death investigations, 55 suspected impaired drivers and 1 sexual assault. The subjects were predominantly female (71%) and had a median age of 40 (mean of 41). The median blood topiramate concentration was 6.2 mg/L (mean 10.8 mg/L, range 1-180 mg/L).

In the subset of death investigation cases, the mean and median age was 40 (range 12 to 63) and 61% were female. The median blood topiramate concentration was 6.6 mg/L (mean 15.2 mg/L, ranged 1.25 to 180 mg/L). At least one other drug was detected in 94% of the death investigations and 91% of the drivers. In one case, an 18 year old, female with one prior suicide attempt, was found unresponsive by her father. She was prescribed topiramate, quetiapine and bupropion for bipolar disorder. Numerous capsules and empty pill bottles were discovered at the scene. Toxicological analysis revealed: topiramate 180 mg/L, quetiapine 34.9 mg/L, bupropion 0.12 mg/L, bupropion metabolite 1.56 mg/L, and atomoxetine 1.55 mg/L. The cause of death was ruled a combined quetiapine and topiramate toxicity and the manner of death was a suicide.

In the driving subset, there was a higher incidence of females (80%) and the median blood topiramate concentration was 6.1 mg/L (mean 6.7 mg/L, range 1 -20.4 mg/L).

One of the driving cases involved a 40-year-old male city bus driver. He had developed a seizure disorder in 1999, had corrective brain surgery in 2001 and was subsequently prescribed topiramate and lamotrigine. He was concerned that topiramate affected his ability to process information, caused him to respond slowly and made multi-tasking difficult, and had complained to his physician. Despite his complaints, his physician wrote a letter in support of his reinstatement as a driver even while trying to wean the subject from his topiramate. In December 2003, the subject struck and killed a co-worker in the bus yard. The driver was evaluated by a drug recognition expert (DRE). During the evaluation he slurred his words and was noted to have coordination and balance difficulties. The DRE conclusion was that the subject was under the influence of a CNS depressant. The toxicological findings revealed lamotrigine concentration of 6.6 mg/L and a topiramate concentration of 3.7 mg/L. What is the conclusion regarding the significance of both drugs here? Any interaction?

Information on blood concentrations of topiramate is scant. This review of 107 cases including both death investigations and suspected impaired drivers found the median blood concentrations to be approximately 6 mg/L in both populations, and identified cases in which topiramate was implicated as the principle causative agent in deaths, and played a role in causing driver impairment.

References:

1. Baselt, R. *Disposition of Toxico Drugs and Chemical s in Man*, 6th ed, Biomedical Publications, Foster City, CA, 2002, pp 1045-1046
2. Mozayani, A, Carter J and Nix R. Distribution of topiramate in a medical examiner's case. *J. Anal Toxicol*. 1999 Oct; 23(6): 556-8.
3. Langman LJ, Kaliciak HA and Boone SA. Fatal acute topiramate toxicity. *J. Anal Toxicol.* 2003 Jul-Aug; 27 (5): 323-4

Topiramate, Death Investigation, Imparied Driver

K38 Drugs in Driving Fatalities in British Columbia, Canada

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Attendees will be briefed on the drugs commonly associated with driving fatalities in BC, Canada.

By understanding the drugs commonly associated with driving fatalities, this presentation will impact the forensic community and/or humanity by helping to design appropriate interventions for target groups.

Objective: Since January 1, 2004, the Provincial Toxicology Centre and the British Columbia Coroner's service have implemented a policy that allows for complete drug screening on all samples collected from driving fatalities. This abstract represents a preliminary review of these results (from January 1, 2004 to June 30, 2004), however, the data presented will cover cases collected from January 1, 2004 until December 31, 2004.

Methods: Toxicology results were included in the study, retrospectively from cases where the deceased was identified as the driver involved in a fatal motor vehicle incident investigated by the BC Coroner's service. Drug screening was performed for illicit drugs including morphine and cocaine and metabolite (COC) and cannabinoids (THC) by immunoassay. Basic drugs were screened by liquid-liquid extraction followed by GC-NPD and GC-MS electron impact detection. Acidic and neutral drugs were screened by liquid-liquid extraction followed by HPLC-DAD. Amphetamine type stimulants (AMP) were screened by LC-MS. Volatiles was assayed by GC-FID.

Results: During the first 6 months of the year there have been 96 driving fatality investigations, where a full drug screen was conducted. The mean age (SD) of cases was 35y (13y), the median 32y, and the range 15 - 66y (N=90). The gender was identified in only 48 of the cases; 38 males and 10 females. Approximately 33% (N=34) of the cases had a negative toxicology screen. The mean age of these cases was 35y (15y), the median was 31y, and the range was 15 - 60y (N=32). The remaining 67% of cases (N=62) had at least one drug identified. The mean age was 35y (12y), the median was 33y, and the range was 16 - 66y (N=58). Of the cases containing drugs, 36 had one drug detected, 15 had two, 7 had three, 2 had four and 2 had five. Ethyl alcohol (EA) was detected most frequently in 58% (N=36) of cases. The mean EA concentration was 0.16 ± 0.10 % (35 ± 21 mmol/L) median 0.18 (39 mmol/L). The following drugs and the mean (SD), median and range of ages is described:

Drug	Age (y) Mean	SD	Median	Range	N=
Ethyl alcohol	33	10	33	16 - 51	36
Cannabinoids	29	12	34	16 - 62	17
Cocaine and metabolite	33	14	30	16 - 66	16
Opiates				23 - 57	7
Amphetamine Type Stimulants				22 - 66	7
Other prescription drugs	37	12	40	16 - 62	28

Of the AMP group, there were 5 cases that contained methamphetamine, one with MDMA, and three with pseudephedrine. One case contained all three. In the opiate containing cases, all had levels of morphine with two of the cases having low levels of codeine, while one case identified MAM. In the cases where EA was identified the most commonly additional identified drugs were COC (N=9), followed by THC (N=5).

Conclusions: The preliminary study indicated that EA is the drug most frequently associated with driving fatalities, followed by THC and COC. There doesn't appear to be a significant difference in the ages of the cases and the different drugs detected. However, due to the relatively small number of cases in the preliminary study, any difference may not yet be apparent. Examination of the data for the year will give a more complete assessment of the demographics of the driving fatalities in BC.

Drugs, Driving, Fatalities

K39 Suicide by Acute Cyanide Ingestion in a 40-Year-Old Male

Douglas E. Rohde, MS, William Bligh-Glover, MD, and Salvatore G. Rizzo, MD, Lake County Coroner's Office, 104 East Erie Street, Painesville, OH 44077; and David A. Engelhart, PhD, and Amanda J. Jenkins, PhD, Cuyahoga County Coroner's Office, 11001 Cedar Avenue, Cleveland, OH 44106*

Attendees will learn of the use of an unusual compound in the commission of suicide at the workplace and the methods used to analyze this compound.

This presentation will impact the forensic community and/or humanity by providing a reminder that autopsy findings in acute cyanide ingestions do not necessarily reveal the nature of the poison and not all toxicology laboratories are equipped to perform cyanide screening. It is therefore essential that the circumstances of the fatality and case history be closely examined.

Ingestion of cyanide is a rapid and effective means of suicide. While cyanide is not readily available in the average place of employment, certain occupations do employ its use and subsequently provide access to this deadly poison. This report describes the autopsy findings and laboratory results of an individual with suicidal intent and occupational opportunity to choose cyanide as the means of his demise.

The deceased was a 40-year-old Caucasian male employed in an electroplating facility where potassium cyanide, silver cyanide, and sodium cyanide were routinely used. According to a supervisor at the workplace, the decedent had a history of depression and had recently exhibited suicidal tendencies. A family member stated that the decedent's brother had committed suicide in 1979. During the morning break period at the electroplating facility, the decedent slipped away unnoticed and was found minutes later unresponsive at his desk. The medical response team initiated CPR and observed a bluish facial color and foaming at the mouth. He was transported to the Lake County Coroner's Office for autopsy. External examination of the body was unremarkable, with notation of an oral endotracheal tube and a 1½-inch irregular laceration of the left posterior parietal scalp. Internal examination revealed severe pulmonary edema. Examination of the gastro-intestinal system gave no indication of esophageal or gastric mucosa inflammation. Microscopic description of the lung indicated intra-alveolar hemorrhage and vascular congestion. No other significant findings were reported.

Routine toxicology testing, including a volatile screen, drugs of abuse screen and general drug screen, was performed on postmortem blood with negative results. Qualitative detection of cyanide was performed using the Merckoquant Cyanide Test [Merck KGA, Darmstadt, Germany] with a blue/lavender color indicating the presence of dissociable cyanide ions. The limit of detection of the qualitative method is 0.2 mg/L. Confirmation and quantitation of positive results was achieved by separation using microdiffusion and measurement with an electrode selective for cyanide ions. A five point curve utilizing 0.5, 2.0, 10, 50, and 100 ppm blood calibrators was generated; correlation coefficient = 0.99556. A low control of 1.0 ppm and a high control of 20.0 ppm were included. The limits of detection and quantitation were determined to be 0.1 ppm and 0.2 ppm, respectively. Linearity was demonstrated from 0.1 – 100 ppm. Analysis of available biological fluids and tissues was performed with the following cyanide results: blood (cardiac) 261.3 mg/L, blood (femoral) 13.7 mg/L, gastric contents 7024 mg/L, urine 1.3 mg/L, vitreous humor 4.5 mg/L, lung 25 mg/kg, liver 6.3 mg/kg, spleen 314 mg/kg. The death was ruled a suicide caused by acute cyanide intoxication.

Cyanide can be detected in the general population in blood concentrations of 0.040 mg/L or lower, with elevated results common in smokers. Reports of suicide in the U.S. by cyanide ingestion are infrequent, and, as this fatality demonstrates, are generally associated with individuals in occupations that employ the use of hydrocyanic acid and its sodium and potassium salts (such as chemists, metal polishers, exterminators, jewelers,

and electroplaters). Autopsy findings in acute cyanide ingestions do not necessarily reveal the nature of the poison and not all toxicology laboratories are equipped to perform cyanide screening. It is therefore essential that the circumstances of the fatality and case history be closely examined.

Cyanide, Suicide, Occupational Opportunity

K40 Fentanyl in Seven Medical Examiner's Cases in the City and County of San Francisco

Nikolas P. Lemos, PhD, Steven B. Karch, MD, Elin Lin, MS, Glenn Nazareno, MD, Venus Azar, MD, Jon Smith, MD, Amy P. Hart, MD, and Boyd G. Stephens, MD, Office of Chief Medical Examiner, Hall of Justice, North Terrace, 850 Bryant Street, San Francisco, CA 94103*

The goal of this presentation is to alert the general community on the apparent rise of fentanyl in medical examiner cases and will also aid forensic toxicologists in the interpretation of postmortem fentanyl levels.

This presentation will impact the forensic community and/or humanity by alerting the general community on the apparent rise of fentanyl in medical examiner cases and will also aid forensic toxicologists in the interpretation of postmortem fentanyl levels.

Fentanyl, a potent opioid analgesic and extremely potent μ agonist, is being detected with increasing frequency in medical examiner cases. At one time, fentanyl abuse was a practice largely confined to medical professionals with ready access. However the advent of the fentanyl patch has drastically changed the situation, and the pattern of abuse is becoming more like that observed with other, less exotic, drugs of abuse. Routes of administration for fentanyl include transdermal, transmucosal/oral, intravenous and combinations of the various routes. There are seven published reports of abusers heating patches and inhaling the vapors. The Office of the Chief Medical Examiner for the City and County of San Francisco serves a population of approximately 750,000 and this number has remained stable for several decades. In 2002, 1,463 cases came under the jurisdiction of the SFOCME; fentanyl was detected in blood, urine and/or tissue of seven cases, giving an incidence of 0.9 per 100,000 people, a rate of detection nearly twice that of MDMA. The data presented herein is the result of a retrospective analysis of all death investigations carried out by the SFOCME, from January 1, 2002 until December 31, 2002. The median age of decedents was 51.3 years (SD=9.0, range 37-71 years). Decedents were overwhelmingly male (86%), and predominantly white (57%). In 2002, the seven deaths were attributed to bronchopneumonia due to chronic polysubstance abuse (2 cases, 29%), complications of acute and chronic cocaine abuse & pulmonary emphysema (1 case, 14%), pulmonary hemorrhage due to complications of end stage renal disease & hypertension and acute and chronic drug abuse (1 case, 14%), asphyxia due to airway obstruction due to carcinoma on the tongue & polypharmacy (1 case, 14%), hypertensive arteriosclerotic cardiovascular disease & acute subdural hematoma (1 case, 14%) and unknown (1 case, 14%). Cocaine was present in two of the seven cases, as was diazepam and hydrocodone. Other drugs present in the postmortem specimens of these seven cases included alcohol, acetaminophen, amphetamine, chlorpherniramine, ibuprofen, methamphetamine, oxycodone, paroxetine, and trazodone. Fentanyl was extracted from biological specimens using liquid-liquid extraction for alkaline drugs and identified and confirmed/quantified by gas chromatography - mass spectrometry (GC-MS) in the electron impact ionization mode. The mean fentanyl blood concentration was 0.03 ± 0.01μg/mL and the mean fentanyl urine concentration was 0.15 ± 0.10 μg/mL. In two cases where fentanyl was measured in the liver, the concentrations were 0.04 and 0.19 μg/g, respectively. Fentanyl was finally quantified in cerebrospinal fluid in one case and the concentration was 0.17 μg/mL. For comparison, a case review from another Medical Examiner's Department where a 55-year-old white female died of an acute fentanyl intoxication complicating treatment for chronic pain (with amitriptyline use listed as contributory cause) with a postmortem blood concentration of 0.02 μg/mL. In that case amitriptyline and nortriptyline were present in postmortem blood at concentrations of 1.1 and 1.1 μg/mL, respectively. These findings show that the City and County of San Francisco just like other areas of the country has experienced a rapidly increasing encountering of fentanyl in medical examiner cases. This may suggest that fentanyl is becoming an additional desired opioid similar to oxycodone and methadone. Expectations are that this study will alert the general community on the apparent rise of fentanyl in medical examiner cases and will also aid forensic toxicologists in the interpretation of postmortem fentanyl levels.

Fentanyl, Postmortem, Polysubstance Abuse

K1 Use of a Novel Large Volume Splitless Injection Technique and Sequential Full Scan/SIM for Simultaneous Screening and Confirmation of Toxicological Specimens

Eric Phillips, BS, Thermo Electron, 2215 Grand Avenue Parkway, Austin, TX 78728*

After attending this presentation, attendees will have the ability to use GC/MS instrumentation to better characterize samples and quantitate unknown compounds.

A novel injection technique coupled with a sequential full scan/SIM acquisition can expand that role and consequently increase the value of GC/MS as an analytical tool. By combining an injection technique that allows injection of up to 35 uL of sample with a sequential full scan/SIM acquisition, the forensic scientist can achieve several objectives with a single injection. SIM analysis enables confirmation of pre-screened presumptive positives, while the presence of full scan data allows investigation into other compounds of interest that may be present in a sample.

The resulting data provided confirmatory, quantitative data about target compounds as well as library searchable results for unknown full-scan peaks. Emphasis was placed on cocaine and metabolites in urine and oral fluid. The custom library contained standards ranging across a broad spectrum of acidic, basic, and neutral drugs. Extraction methods were appropriate for the target compounds. The limit of detection using a standard splitless injection technique was 30 ng/mL for benzoylecgonine (BE) in urine, and this LOD was decreased to 300 pg/mL using an injection volume of 35 uL. The correlation coefficient for BE in urine using the SIM data was 0.991, calibrated from 300 pg/mL to 1200 ng/mL. Run times using the large volume technique were longer than those using the standard technique, due to use of a longer column and a required period of isothermal oven temperature. These longer run times were offset by the decreased detection limits achievable with the large volume injection.

Large Volume Splitless Injection, GC/MS, Sequential Full Scan/SIM

K2 Urinary Fentanyl and Norfentanyl During Application of Duragesic® Transdermal Patches

Alphonse Poklis, PhD, Department of Pathology, Virginia Commonwealth University, Richmond, VA 23298-0165; Ronald C. Backer, PhD, Ameritox, 9930 West Highway 80, Midland, TX 79706*

After attending this presentation, attendees will have data concerning the urinary concentration of fentanyl and its major metabolite norfentanyl in patients treated with “Duragesic®” transdermal patches.

Toxicologist will be aware that urine concentrations of fentanyl and norfentanyl in patients on Duragesic® transdermal patches for control of chronic pain will far exceed previously reported values from overdose cases or patients receiving fentanyl for control of acute pain.

This poster presents the urinary concentration of fentanyl (F) and it’s major metabolite norfentanyl (NF) in chronic pain patients treated with “Duragesic” transdermal patches. The Duragesic® continuous release transdermal patch is designed to release 25 µg/h per 10 cm^2 of surface area. The patches are available in 10, 20, 30 and 40 cm^2 sizes releasing 25, 50, 75 and 100 µg/hr F, respectively. Desired therapeutic blood concentrations are obtained 8 to 12 hours after patch application. F is rapidly and extensively metabolized, with NF as the major metabolite. Little data is available on expected urine F concentrations in therapeutic situations, while urine concentrations in overdose cases have been reported to range from 5-93 ng/ml (Baselt, 1995).

Random urine specimens were collected from 200 chronic pain patients wearing 25, 50, 75 or 100 ug F transdermal patches. Urine specimens were collected from hours after application to several days later after continuous F release. Each specimen was analyzed for F, NF, creatinine and pH. Additionally, each was screened by enzyme immunoassay for the following: amphetamines, barbiturates, benzodiazepines, cocaine metabolite, methadone, phencyclidine, d-propoxyphene, opiates and marijuana metabolites. All positive screening results were confirmed by GC/MS. F and NF were isolated from urine by solid phase extraction (Biochemical Diagnostics GV-65), then identified and quantified by GC/MS in SIM mode. Mass to charge ions monitored were: F, 245,146, 246; d_5-F, 250, 251; NF-acetyl derivative, 231,158,132; and d_5-NF-acetyl derivative 236. Quantification of F and NF was by comparing ions 250/245 and 236/231, respectively. The LODs and LOQs for both F and NF were 3 ng/mL. The ULL for F and NF were 250 and 400 ng/mL, respectively. The results of F and NF analysis are presented below:

Dose, ug/hr		Fentanyl ng/mL		Norfentanyl ng/mL	
Patch	No.	mean	range	mean	range
25	46	48	0 - 474	161	0 -800
50	71	78	0 - 569	222	0 - 931
75	29	75	0 - 444	245	0 - 820
100	51	159	0 - 631	220	0 - 722

The incidence of other drugs detected as a percentage the 200 specimens were: opiates, 48% (incidence: Codeine 1, Hydrocodone 41, Hydromorphone 7, morphine 12, oxycodone 36); benzodiazepines, 43%; barbiturates, 3%; methadone, 4%; marijuana metabolite, 3%; amphetamines, 2% and cocaine metabolite, 1%. Other than F and/or NF no drugs were detected in only 25% of the specimens.

Conclusion: These data demonstrate the wide variation in concentrations of F and NF in random urine specimens following application of Duragesic® patches. However, these values obtained during therapeutic use far exceeded concentrations previously reported in fatal poisoning. In general, one may expect to find urine NF concentrations 3 to 4 times higher than those of F. Also, in addition to fentanyl therapy, chronic pain patients routinely are prescribed other opiates and benzodiazepines.

Fentanyl, Norfentanyl, Urine Drug Testing

K3 Use of the QED® Saliva Enzymatic Alcohol Test Device for the Identification and Quantitation of Alcohol in Urine

John Vasiliades, PhD, Kathy Lewis, and Kristin Colonna, BS, Toxicology Labs, Inc., 4472 South 84th Street, Omaha, NE 68127*

After attending this presentation, attendees will learn the use of the QED® Saliva Enzymatic Alcohol Test Device for the identification of ethanol in urine and to determine the concentration of ethanol in urine.

Method: The QED® Saliva Alcohol Test (STC Technologies, Inc., Bethlehem, PA 18018) is a rapid enzymatic alcohol dehydrogenase assay

which quantitatively measures alcohol concentrations from 0 to 150 mg/dL. We evaluated the QED® A-150 Saliva Alcohol Test Device for the determination of alcohol in urine. We followed the manufacturer's procedure, except that the cotton tip of the swab was dipped into urine so that the cotton swab was saturated with urine. Samples were analyzed on the same day by Gas Chromatography (GC) with flame ionization detector (FID) on a glass column, 1.82 m x 2 mm ID glass column, 60/80 Carbopack B/ 5% Carbowax 20M (Supelco, Bellefonte, PA, 16823). N-propanol (NP) is used as internal standard (IS). Urine samples, which were spiked with ethanol at 20, 40 and 80 mg/dl gave the following average results. Within-run precision by QED® at the 3 concentrations (n=12) was 7.3% with a 128 +/- 31% recovery; between-run precision averaged 11% with 131 +/- 29% recovery. For comparison the average within-run precision by GC at the 3 concentrations (n=12) was 2.9% with a 104 +/- 5% recovery; between-run precision averaged 4% with 103 +/- 3% recovery. Urine samples that were analyzed on the same day by QED® and GC gave the following results. The concentration of samples ranged from 0 to 383 mg/dl of ethanol with a mean of 117.35 and standard deviation (+/-) of 79.01 by GC mean= 117.35 +/- 79.01, n=31) and a mean of 100.09 and standard deviation (+/-) of 65.75 by QED® (mean= 100.09 +/- 65.75, n=31). Least squares analysis of urine alcohols by GC (x) in comparison to QED® (y) gave a slope (m) of 0.929, y-intercept (b) of -1.028 and correlation coefficient (r) of 0.99 (y = 0.929x - 1.028, r= 0.99) with a standard error of estimate Syx of 14.95. Recovery studies indicate that QED® overestimates urine alcohols at low concentrations. No false positive results were reported by QED®. Interference studies indicate that n-propanol will cross react 60% and isopropanol 20% with the QED alcohol method. We conclude that the QED® saliva method can be used for the determination (identification and quantitation) of alcohol in urine. Although QED does not have the sensitivity, selectivity and precision or accuracy of GC, it will provide qualitative and quantitative results more rapidly than GC, less than 3 minutes.

Urine Alcohol, Gas Chromatography, QED®

K4 Analysis of Amphetamines in Nail Clippings Collected From Female Prisoners

Dong-Liang Lin, PhD, Rea-Ming Yin, BS, and Hsiu-Chuan Liu, BS, Institute of Forensic Medicine, No. 16, Lane 175, Tong-Hwa Street, Taipei 106, Taiwan*

This presentation will demonstrate the usefulness of fingernails as an analytical specimen for confirming amphetamine use and the relationship of evidence of amphetamines in hair and nail specimens

With respect to the use of fingernail as an analytical specimen, fewer studies have been directed to amphetamines than other commonly abused drugs, such as opiates or cocaine. In this study, paired fingernail and hair specimens were collected from 43 consenting female prisoners who have admitted the use of amphetamines and/or opiates. These specimens were quantitatively analyzed for amphetamine, methamphetamine, methylenedioxyamphetamine, and methylenedioxymethamphetamine. Methamphetamine and amphetamine concentration ranges, and methamphetamine/amphetamine ratios found in the 21 amphetamines-containing specimens were 0.46-58.17 ng/mg, <0.20-5.42 ng/mg, and 4.06-14.01, respectively. Six paired hair specimens from these 21 sets were selected and cut into 1.5-cm sections. The first 5 sections (from the root) were analyzed. Analytical data are shown in Table 1.

Table 1. Amphetamines in fingernail and hair

Sample No.	Fingernail (ng/mg)			Hair (ng/mg)			
	Methamph.	Amph.	Methamph./ Amph.		Methamph.	Amph.	Methamph./ Amph.
3 (A-008)	13.96	2.73	5.11	S-1[a]	16.78	4.32	3.88
				S-5[a]	58.78	12.83	4.58
5 (A-013)	12.43	1.70	7.31	S-1	18.95	2.27	8.35
				S-5	38.29	3.59	10.67
8 (A-024)	58.17	5.42	10.73	S-1	134.1	24.37	5.50
				S-5	80.55	10.42	7.73
11 (A-027)	3.94	0.97	4.06	S-1	7.03	1.76	3.99
				S-5	30.23	5.89	5.13
13 (A-030)	43.63	3.38	12.91	S-1	71.81	11.59	6.20
				S-5	9.24	1.73	5.34
19 (A-041)	11.70	1.42	8.24	S-1	20.95	3.84	5.46
				S-5	45.25	6.44	7.03

[a] S-1, S-5: The first and the 5th sections of the 5 sections analyzed.

It is interesting to note that results obtained from hair sectional analysis follow definite trends. Specifically, the concentrations of methamphetamine and amphetamine in samples 3, 5, 11, and 19 increase continuously, while the same analytes' concentrations in samples 8 and 13 decrease continuously. Nail clippings will be continuously colleted on biweekly intervals. Whether the analytes' concentrations in nail specimens will follow the same trends observed for hair will be investigated.

Nail, Amphetamines, Hair

K5 Validation of Volatile Analysis Using Dual Column Capillary GC

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The authors will present data obtained during the validation of a dual column capillary gas chromatography (GC) procedure. The assay, which is routinely used by the New Mexico Department of Health for evidential ethanol testing and postmortem investigation, was validated in terms of precision, accuracy, matrix effects, carryover, linearity, limit of detection and limit of quantitation. A comparison of quantitative ethanol concentrations using postmortem and antemortem casework using both capillary columns (Restek BAC1 and BAC2), together with a comparison of capillary and packed GC columns is also described.

A targeted analysis is performed for methanol, ethanol, acetone and isopropanol using an Agilent HP 6890 GC equipped with a flame ionization detector (FID). Methanol, ethanol, acetone and isopropanol are identified based upon characteristic retention times relative to the two internal standards, n-propanol and t-butanol.

The limit of detection (LOD) in blood was 0.001 g/dL for all analytes tested. The limit of quantitation (LOQ) for ethanol, isopropanol and acetone was 0.005 g/dL and 0.010 g/dL for methanol. Precision using whole blood was evaluated by replicate analysis of in-house controls (n=8). Intraassay CVs for ethanol, methanol, acetone and isopropanol were 1.1, 1.1, 1.0 and 1.1% at 0.474 g/dL, 1.2, 0.9, 1.5 and 0.8% at 0.158 g/dL, 1.7, 1.6, 2.4 and 1.2% at 0.079 g/dL and 4.4, 3.5, 1.9 and 3.4% at 0.019 g/dL respectively. Intraassay CVs using a commercial whole blood control (BioRad) were in the range 2.2 - 3.1% (n=8). Accuracy was determined using internal and external controls. Accuracy using in-house blood controls was 99-103% in the concentration range tested (0.039 – 0.379 g/dL). Accuracy using aqueous external controls (Cerilliant) was 96-102% and commercial whole blood controls (Utak Laboratories, BioRad) were within the acceptable limits defined by the

manufacturer. Analysis of samples fortified with compounds at concentrations that were unknown to the analyst revealed concentrations of ethanol, methanol, acetone and isopropanol within 95 - 105% of the target concentration. No matrix effects were observed and the calibration was linear at 0.7 g/dL, the highest concentration tested. No carryover for any of the analytes was detected at this level. Quantitative ethanol concentrations in 128 postmortem and antemortem case samples were compared using both capillary columns. Linear regression analysis revealed an R2 value of 1.000 (y = 1.0138x – 0.0003), where BAC1 and BAC2 were plotted on the y and x-axis respectively. The mean interassay CV was 1.48% for casework samples that contained ethanol concentrations in the range 0.010 – 0.367 g/dL. Quantitative results using the new capillary GC procedure were compared with a previously used packed GC-FID procedure. Analysis of 108 postmortem casework samples revealed an R2 value of 1.000 (y = 1.0188x – 0.00009), where blood ethanol concentrations using capillary and packed GC columns were plotted on the y and x-axis respectively. The mean interassay CV was 1.41% for casework samples that contained ethanol concentrations in the range 0.010 – 0.277 g/dL.

Headspace Analysis, Ethanol, Capillary GC

K6 An LC-Electrospray Tandem Mass Spectrometric Method for Identification and Quantitation of Cocaine and Key Metabolites in Biological Matrices

Jason E. Schaff, PhD and Marc A. Lebeau, MS, FBI Laboratory, Chemistry Unit, Room 4220, 2501 Investigation Parkway, Quantico, VA 22135*

After attending this presentation, attendees will understand a highly sensitive and specific method for analysis of cocaine and its primary metabolites in blood and urine

Since cocaine is one of the most widely abused illegal drugs, providing a robust and reliable new method for the analysis of this compound and its metabolites will likely prove valuable to many practitioners in the fields of criminal forensic toxicology, workplace and sports drug testing, and postmortem toxicology. Many features of the reported method help to ensure that accurate testing results are obtained rapidly with a high degree of analytical confidence.

This poster will present a recently developed method for the simultaneous analysis of cocaine, benzoylecgonine, methylecgonine, and cocaethylene in biological matrices. Both validation data and data from several cases illustrative of the analytical power of the method will be presented.

The presented LC-ESI-MS/MS analytical method was developed to replace an existing GC-MS (CI) method that relied upon derivatization of the extracted analytes to achieve sufficient component volatility. Prior experience had revealed several limitations to this method, including poor long-term stability of the derivatives, poor derivatization efficiency in some putrefied samples, and compromised chromatography in many putrid or highly concentrated specimens. Additionally, the derivatizing reagents for the GC-MS method were costly, toxic, and had a relatively short shelf life, making their elimination highly desirable.

The solid phase extraction of specimens from the prior method was retained essentially intact, with only a change in one of the internal standard compounds. The new internal standard for benzoylecgonine was the phenyl-d5 isotopomer, which, in combination with the use of d3-cocaine, provides a built-in check for any in-assay hydrolysis. In the new method, dried extracts were reconstituted in unbuffered mobile phase and analyzed directly by LC-ESI-MS/MS, using a column and isocratic mobile phase from a specialty method already in use for trace-level quantitation of benzoylecgonine in solid tissues.

The new analytical system was validated on a series of blood calibration curves over a two order of magnitude concentration range for each component. Curves were extracted on three separate days and analyzed in duplicate, with one curve also rerun a day later to check for compound stability. Analytical run time was 15 min, comparable to the 11.5 min run time from the prior GC method. Validation results were generally very good, with excellent lower limits of detection and quantitation, good between day reproducibility, wide linear ranges, and negligible carryover. Best quantitative results were obtained by measurement of RIC traces for the pseudomolecular ions for the analytes and internal standards in full scan MS mode, while tandem mass spectrometry provided unambiguous identification of all analytes. Analysis was performed to a data-dependant scanning mode to allow collection of both types of data in a single analytical run. One interesting cautionary observation emerges from these data. With the chosen chromatographic system, methylecgonine is separated from its N-methyl-d3 isotopomer by almost one minute.While not unheard of, this is a very rare observation, and one that many forensic scientists may not typically have encountered in practice.This peak separation does somewhat compromise the quantitative analysis of methylecgonine, which shows the smallest linear range of the four targeted compounds.

Several of the case specimens analyzed since the development and adoption of this new method illustrate its great power and stability. To date, no sample has been able to "break" the method from the standpoints of putrefaction, interferences, carryover, or failed recovery of internal standards. The only cases requiring reanalysis have resulted from analyte concentrations higher than the chosen calibration range.

The authors feel that this reliable and robust analytical method will be of value to many forensic toxicology laboratories, and points towards a scheme for potential improvements in many other targeted toxicological analyses.

Cocaine, Liquid Chromatography, Mass Spectrometry

K7 Use of the Covino Algorithm in Evaluating the Additive Toxicity of Lidocaine® and Bupivacaine

David M. Benjamin, PhD, Tufts Medical School, 77 Florence Street, Suite 107, Chestnut Hill, MA*

This research project are discuss how to convert bupivacaine blood levels to lidocaine equivalents so that the blood levels of both drugs can be used to assess toxicity in combined lidocaine/bupivacaine toxicity.

The local anesthetics (LAs) lidocaine® and bupivacaine are commonly used together in many surgical and cosmetic procedures. Routes of administration run the full gamut and include: intravenous (IV) lidocaine® as an antiarrhythmic, local infiltration for surgical repair of lacerations and during "face lifts", extensive infiltration for liposuction, and epidural and subarachnoid administration for abdominal and lower extremity surgery. LA administration may be either the sole analgesic employed, or may be combined as an adjunct with general anesthesia. However, local anesthetics are not innocuous and serious central nervous system (CNS) and cardiovascular toxicity can result in seizures, cardiovascular collapse and death. Antiarrhythmic levels of lidocaine® range from 2-5 mcg/ml and toxic effects are well-established in the 6-10 mcg/ml range; however, the same relationship is less well understood for bupivacaine, and evaluation of blood levels for mixed lidocaine®/bupivacaine toxicity is poorly understood as evidenced by the following case report.

Facts of the case: RB was a 60-year-old female in good general health who underwent facial cosmetic surgery and subsequently died from cardiorespiratory collapse. She had been diagnosed with depression and was taking Fluoxetine (Prozac®) and no other medications. The medical examiner's report stated the following: The intubation went smoothly and she was initially stable from a cardiovascular point of view. About five minutes into the anesthesia a local anesthetic consisting of lidocaine, bupivacaine and epinephrine was injected beneath the skin of the face and scalp. She began to demonstrate cardiovascular instability …. Heart rate decreased to the 30s and CPR was begun with return of pulse and blood pressure. She again deteriorated and was transferred by paramedics to the ER…. Her condition gradually deteriorated to anoxic encephalopathy and she was pronounced dead approximately seven hours after the initial cardiovascular collapse. Toxicology ante- and postmortem lidocaine® and bupivacaine blood levels were:

Time	Ante-Mortem Blood Concentrations (mcg/ml)		Post-Mortem Blood Concentrations (mcg/ml)	
	Lidocaine®	Bupivacaine	Lidocaine®	Bupivacaine
9:50 a.m.	3.1	0.9		
10:34 a.m.	4.5	2.0		
4:27 p.m.		DEATH		
At autopsy: (46 hours after death)			5.3	3.3

Injection sites were identified as follows: "On the midline upper forehead just below the hairline is a punctuate mark representing a needle puncture. A second punctuate mark is present on the left lower forehead just above the lateral end of the left eyebrow. A third punctuate mark is noted on the left upper lateral cheek just lateral to the lateral angle of the left eye. Fourth and fifth punctures are noted on the lateral aspect of the right eye…. A ½ inch area of subcutaneous hemorrhage is present about the lower of the two puncture marks near the lateral angle of the right eye." The Medical Examiner also offered the following interpretation in his report, "Lidocaine® and bupivacaine levels are within the range previously documented following clinical administration. The actual mechanism of her cardiorespiratory collapse is unknown."

Benjamin G. Covino, MD, PhD, was Professor of Anesthesiology at Harvard Medical School and Vice-President of Astra Pharmaceuticals, (a company that marketed lidocaine® and bupivacaine) from the mid-1960s through the 1980s and was an internationally-respected expert in local anesthesia pharmacology and toxicology. Dr. Covino recognized that bupivacaine was four times as potent as lidocaine® and that toxic blood levels of lidocaine® ranged from 6-10 mcg/ml while those of bupivacaine ranged from 1.5-2.5 mcg/ml. Dr. Covino was also the first investigator to convert blood levels of bupivacaine to "lidocaine® equivalents" by multiplying the bupivacaine blood level by four and adding that number to the blood level of lidocaine® to obtain the combined blood level of both local anesthetic agents in "lidocaine® equivalents". Applying the "Covino Algorithm" to the above data, at 9:50 am, a lidocaine® equivalent level of 6.7 and at 10:34 am, a lidocaine® equivalent level of 12.5, well into the toxic cardiodepressant level, and rising. The rapid appearance of bradycardia most likely indicated an initial unintended intravascular administration followed by continuous absorption from the infiltrated region resulting in an additive toxic cardiovascular effect that was further compromised by poor management and hypoxemia, and resulted in the death of a patient.

Lidocaine®, Bupivacaine, Additive Toxicity

K8 Distribution and Optical Purity of Methamphetamine Found in Toxic Concentration in a Civil Aviation Accident Pilot Fatality

Patrick S. Cardona, BA, Arvind K. Chaturvedi, PhD, John W. Soper, PhD, and Dennis V. Canfield, PhD, Bioaeronautical Sciences Research Laboratory (AAM-610), FAA Civil Aerospace Medical Institute, PO Box 25082, Oklahoma City, OK 73125-5066*

The authors will present toxicological findings of a pilot fatality involved in a unique methamphetamine-related civil aviation accident to aid investigations of such accidents.

This presentation will provide information on the distribution of methamphetamine present in toxic concentration and the stereoselective analysis of this amine in biological samples.

The Federal Aviation Administration's Civil Aerospace Medical Institute conducts toxicological evaluation of postmortem biological samples collected from pilots involved in fatal civil aircraft accidents. The submitted samples are primarily analyzed for the presence of primary combustion gases, alcohol/volatiles, and drugs. Related to such an evaluation, findings of a unique aircraft accident are described in this report. Upon colliding with terrain in weather conditions of poor visibility, a 1-occupant airplane was substantially damaged with no evidence of fire. Remains of the pilot were found outside the crashed aircraft. Pathological examination of the pilot's body revealed multiple blunt force injuries and vascular congestion, including subdural hemorrhage of the cerebral cortex. Autopsied samples—blood, brain, gastric contents, heart, liver, muscle, spleen, urine, and vitreous fluid—were submitted for toxicological analysis. The fluorescence polarization immunoassay disclosed the presence of 8.0 µg/mL amphetamines in urine. Subsequent gas chromatographic/mass spectrometric confirmatory analysis determined the presence of methamphetamine (1.134 µg/mL in blood and 59.171 µg/mL in urine) and amphetamine (0.022 µg/mL in blood and 1.495 µg/mL in urine). Both amines were present in all the submitted sample types, except for amphetamine, which was detected neither in vitreous fluid nor in muscle. The amount of methamphetamine found in gastric contents was 575-fold higher than that of amphetamine. Stereochemical analyses of gastric contents, blood, and urine using a chiral probe, (S)-(–)-N-(trifluoroacetyl)prolyl chloride, indicated that methamphetamine detected in the sample types was not optically pure. In gastric contents and urine, this secondary amine's optical isomers were present in equal proportions. The enantiomeric excess of (+)-methamphetamine over its (–)-form was about 32% in blood. Both optical forms of amphetamine were present in the ratio of 1.2–1.5:1.0 in the 3 sample types. The blood methamphetamine concentration found was in the range sufficient to produce toxic effects, including performance impairment. The observed variation in the ratios of amine isomer concentrations in the sample types would have been attributed to stereoselective metabolic and other pharmacokinetic processes. Findings of this study supported the conclusion of the National Transportation Safety Board that, in addition to the visibility-associated adverse meteorological conditions, the use of the controlled substance played a contributory role in the causation of the aircraft accident.

Forensic Toxicology, Methamphetamine, Stereochemical Analyses

* Presenting Author

K9 An Analytical Protocol for the Identification of Sildenafil (Viagra®) in Specimens Commonly Submitted to the Toxicology or Analytical Laboratory

Jennifer S. Button, BSc, Nikolas P. Lemos, PhD, John Ramsey, Terry Lee, and David W. Holt, DSc, Forensic Toxicology Service, St. George's Hospital Medical School, University of London, London, England SW17 0RE, United Kingdom*

Attendance at this presentation will enable the participant to learn a new analytical protocol for the determination of sildenafil (Viagra®) in specimens commonly submitted to the toxicology or analytical laboratory such as clinical biological specimens or tablets suspected to contain this substance.

As licit and illicit sildenafil use is on the increase due the ease with which this substance may be purchased via the internet, it is important for laboratories to have methods for its detection in unknown tablets or in biological specimens of those suspected or known to be using it for recreational or therapeutic purposes. This paper's contribution to the forensic community is that it offers a variety of analytical protocols for the identification of sildenafil.

Our Forensic Service offers a screening and quantification toxicology service to most of Her Majesty's Coroners and Forensic Pathologists in London as well as various Police Forces and one branch of the Armed Forces. As a result, we are required to screen for a large number of prescribed and illicit drugs in ante- and post-mortem specimens followed by quantification of those detected. All analyses must be completed and our final report must be submitted to the Courts within 15 business days of the arrival of the case at the Service. In addition, we provide a tablet and capsule identification service to national and international clients using the commercially available product, TICTAC (www.tictac.org).

The methodology developed involves the detection of sildenafil in unknown tablets confiscated by the Police, purchased on the Internet or voluntarily surrendered by the public. Methods used included thin layer chromatography (TLC), UV-Visible spectrophotometry, gas chromatography – mass spectrometry (GC-MS) and high performance liquid chromatography – tandem mass spectrometry (HPLC-MS-MS). Each unknown tablet was weighed and approximately 10mg were removed from its core, dissolved in 10mL of methanol or 10mL of deionized water and sonicated for 45 minutes in an appropriately-labeled glass tube. The methanolic tablet mixture was centrifuged and approximately 10µL of the supernatant were spotted onto a silica bonded TLC plate alongside a standard mixture containing five standards (amitryptiline, dextropropoxyphene, methaqualone, morphine and nicotine) and a known positive sildenafil control prepared using the pure analyte of interest generously provided by the drug manufacturer, Pfizer Ltd. The TLC mobile phase consisted of methanol and ammonia (v/v 100:0.5). The developed plates were visualized under UV illumination and spots including those of the known and suspected sildenafil fluoresced. After spraying the developed plate with neutral and acidic iodoplatinate reagent, sildenafil produced a visible strong purple and brown colored spot, respectively. The response factor, Rf, for sildenafil was 0.66 whereas those for methaqualone, dextropropoxyphene, nicotine, amitriptyline and morphine were 0.85, 0.69, 0.58, 0.48 and 0.36, respectively. Each aqueous tablet mixture was adjusted to acidic or alkaline pH and subjected to UV-Visible spectrophotometry from 400 to 190nm. Very characteristic spectra were produced with strong absorbances noted at 290nm and 210nm. 1mL of each methanolic tablet mixture was added to 1mL of methyl tert-butyl ether (MTBE) and 100µL of internal standard (quinoline, pyribenzamine, flurazepam, 1mg/mL, respectively), mixed, centrifuged and an aliquot of the resulting supernatant (1µL) was injected onto a GC-MS comprising of the HP 5890 GC coupled to an HP 5971 MS. The analytical column used was Solgel (30m x 0.25mm i.d., 0.25µm film thickness). The injector was maintained at 250°C, the detector was maintained at 280°C and the column temperature program started at 70°C for 4 min, ramped 40°C/min and held at 280°C for 50.75 minutes giving a total run time of 60 minutes. Each unknown tablet together with positive and negative controls was screened in full scan mode and sildenafil was identified by its retention time (R_t), which measured 48.5 minutes. Each methanolic tablet mixture was further diluted in 80% aqueous methanol to an approximate final concentration of 0.1mg/L and 20µL were injected onto an HPLC-MS-MS. The ions monitored were 472.9 and 281.9. The total run time per sample was 3.5 minutes with sildenafil eluting at 2.4 minutes. The analytical column used was a 15cm x 4.6mm i.d., Supercosil LC-18-DB (5µm particle size) ODS column maintained at 50ºC using a Perkin Elmer series 200 column oven. Isocratic solvent delivery was achieved using a Perkin Elmer series 200 pump set at 1mL/min. Sample injection, 20µL, was performed by a Perkin Elmer series 200 auto-injector. The mobile phase consisted of methanol/water (v/v 85:15) supplemented with ammonium acetate solution to achieve a final concentration of 2mmol/L. Detection was by tandem mass spectrometry (HPLC-MS-MS), using a Sciex API 2000 triple quadrupole mass spectrometer (Applied Biosystems). A turbo ion spray (heated electrospray) source heated to 300°C was used to introduce the sample into the mass spectrometer. A post-column splitter (10:1) was installed just before the ion spray interface. The mass spectrometer was operated in positive ionization, multiple reaction mode (MRM, MS-MS), with the resolution set to unit resolution (± 0.5m/z). High purity air was used as the nebulizer gas and high purity nitrogen as the collision gas. The Applied Biosystems Sciex Analyst software was used to control the HPLC-MS-MS, record the output from the detector, integrate and calculate peak areas. In assays requiring quantification, the Analyst software was used to calculate the peak area ratios, produce the calibration line using $1/x^2$ weighed through zero regression and to calculate the concentration of sildenafil.

Finally, the HPLC-MS-MS analytical protocol was successfully used to screen post-mortem human blood from cases in which sildenafil was suspected to be involved. To compensate for the lack of a suitable internal standard, analytical standards were prepared and analyzed in duplicate in deionized water and added to 100µL of sildenafil-free human blood. 100µL of the case blood specimen were added to 100µL of deionized water. 250µL of phosphate buffer (pH 7.0) and 1mL of MTBE were added to the case specimen and standards. The solutions were then mixed for 5 minutes and centrifuged at 3500rpm for 5 minutes. The supernatant for each tube was collected and evaporated to dryness using a Savant SpeedVac SC200 coupled to a Savant RT4104 refrigerated condensation trap. The residue was then reconstituted in 250µL of 80% aqueous methanol, vortex mixed for 30 seconds and injected onto the HPLC-MS-MS using the analytical protocol described above.

As licit and illicit sildenafil use is on the increase due the ease with which this substance may be purchased via the Internet, it is important for laboratories to have methods for its detection in unknown tablets or in biological specimens of those suspected or known to be using it for recreational or therapeutic purposes.

Sildenafil, Viagra®, Identification

* Presenting Author

K10 Determination of Opiates (Morphine) in Postmortem Bone Marrow and Comparison With Blood Morphine Concentrations

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After attending this presentation, attendees will know how long after a body postmortem buried could be analyzed for opiates.

The aim of this presentation is to predict how long after a body postmortem buried could be analyzed for opiates. In sudden, unexpected deaths, traumatic or pathologic mechanism must be excluded as possible causes of death and selection and preservation of appropriate specimens for toxicologic analyzes is very important. For toxicologic analyses generally used specimens are blood and urine. Frequently forensic toxicologist is faced with situations in which contamination and decomposition make the collection of blood samples impossible for suitable analytical purposes.

Material and Method: In such cases, to prove the viability of bone marrow analyzes, to 9 albino rabbits were injected morphine into the marginal ear vein in 0.1 mg/kg increased doses from 0,3 gram/kg for each rabbit. One hour after dosing, the rabbits were sacrificed and blood, urine and bone marrow samples were collected for analyzes. The whole body with other extremities bones were buried. At the seventh and fourteenth day, the bones were excavated and bone marrow specimens were collected. CEDIA® technology was used for the analysis.

Results and conclusion: It was demonstrated that an increase in the given total morphine dose and morphine dose per kilogram of cases, an increase blood morphine concentration also occurred. In urine samples, high morphine doses were detected. There were no quantitative linear relationship between the concentration of morphine in urine and blood. In comparison of blood morphine concentration to postmortem [immediately collected] bone marrow morphine concentration, there were statistically meaningful increase in bone marrow morphine concentrations with increasing of blood morphine concentration. Also the increasing of blood morphine concentration was correlated with postmortem seventh and fourteenth day bone marrow morphine concentrations. Morphine concentration seventh and fourteenth day bone marrow decreased relative to the postmortem [immediately collected] bone marrow morphine concentrations.

In drug related sudden deaths. Drugs cannot be determined in resistant tissues like hair and in cases of contamination and decomposition exclude the collection of blood or in skeletal remains, bone marrow may be the most available evidence. It's concluded that further experimental research in this area should be useful and indispensable for forensic toxicology.

Postmortem, Bone Marrow, Opiates

K11 Improving Data Collection Procedures for the Fatal Analysis Reporting System (FARS)

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Participants will learn about 1) the data contained in the FARS (Fatal Analysis Reporting System) database and how to access the data online, 2) how the data is collected and analyzed, and 3) what measures can be implemented to improve the data collection process.

The National Highway Traffic Safety Administration (NHTSA) has collected extensive data associated with fatal traffic accidents in the FARS database since 1975. Over 100 data elements are coded from sources including the police accident reports, death certificates, EMS reports, and coroner/medical examiner reports. Data is collected from the 50 states, the District of Columbia, and Puerto Rico by FARS analysts who code and transmit the data, in a standard format, to the National Center for Statistics and Analysis. The FARS database can be accessed at www-fars.nhtsa.org.

Toxicological data in the database consists of quantitative alcohol results and qualitative results for drugs other than alcohol. Based on the data submitted, estimates are made of the number of alcohol involved fatal traffic accidents for the nation, and on a state-by-state basis. Data submission rates vary significantly from state to state. In 2001, 16,653 fatally injured drivers nationwide had blood alcohol concentration (BAC) results reported to FARS out of a total of 25,840, or 64%. However, only 33 percent of the fatally injured drivers in Texas had BAC results reported compared to 81 percent reported in California.

An informal poll of medical examiner toxicologists indicates the problem is not a lack of testing for alcohol in deceased drivers. Most medical examiner offices test all drivers involved in a fatal traffic accident for alcohol. The poll did indicate most offices are not aware of the FARS database and the need to provide toxicological test results. An electronic data transfer system for submitting data directly from the forensic toxicology laboratory to the Texas Department of Public Safety and the FARS data collection analysts will be presented.

FARS, Traffic, Toxicology

K12 Rapid Blood Alcohol Analysis With a New Automated Headspace-Gas Chromatographic System

*Roger L. Firor, PhD**, Agilent Technologies, 2850 Centerville Road, Wilmington, DE 19808; Manuela Bergna, Dani Instruments S.p.A., Viale Brianza, 87, 20093 Cologno Monzese (MI), Milan, Italy; Philip Wylie, PhD, Agilent Technologies, 2850 Centerville Road, Wilmington, DE 19808*

After attending this presentation, attendees will have knowledge of state-of-the-art hardware and methods for performing high throughput blood alcohol analyses.

The determination of blood alcohol levels is one of the most frequent analyses performed in forensic toxicology laboratories. In particular, results by gas chromatography are widely respected by the courts.

A new automated 70-sample headspace sampler has been developed utilizing an inert flow path for improved sample repeatability, accuracy, and reduced carryover. These and other improvements in the headspace hardware reduce the frequency of calibration and performance check samples. High sample throughput is possible with the combination of optimized sample heating overlap and new capillary columns developed for rapid separation. Complete GC blood alcohol analysis in under 4 minutes with cycle times of less than 7 minutes are achieved while maintaining baseline separation of methanol, ethanol, isopropanol, n-propanol, and n-butanol. Additionally, the system will resolve many other common interferents like acetaldehyde and acetone.

Headspace control software will be described that integrates headspace parameters and sequencing into the chromatographic data system. Detailed information on sample vial number and result logging essential for proper tracking of samples is included.

Blood Alcohol, Headspace, Gas Chromatography

K13 Laboratory Analysis of Remotely Collected Oral Fluid Specimens for Ethanol by Enzymatic Assay

Dean F. Fritch, PhD, Suzanne Fasolka, BS, Keith Kardos, PhD, Lisa Yeager, BS, Tiffany Fries, BS, Bonnie Martinez, PhD, Eileen Sevem, BS, and R. Sam Niedbala, PhD, Orasure Technologies, Inc., 150 Webster Street, Bethlehem, PA 18015*

Currently, the drugs of abuse that are available from Orasure Technologies, Inc. for testing with the microplate ELISA assays do not include ethanol, which is one of the most abused drugs. This poster describes an enzymatic assay for ethanol that can be performed in a 96 well microplate which can be analyzed using the same equipment used for the analysis of the other drugs of abuse by ELISA.

This should enable laboratories to analyze ethanol in a 96 well plate format along with the other Intercept microplate assays to improve throughput and decrease sample handling, thereby decreasing chances of error.

The performance characteristics of this method for detecting ethanol in oral fluid specimens using a qualitative, enzymatic assay were examined. The assay uses oral fluid which is obtained using the Intercept® DOA Oral Specimen Collection device. The Intercept® DOA Oral Specimen Collection device utilizes a collection pad which collects approximately 0.4mL of oral fluid. The collection pad is then placed in a vial containing 0.8mL of a buffered preservative solution, which is then shipped to the laboratory for analysis. The laboratory then centrifuges the device to collect the diluted oral fluid mixture. Sample from the device is added to a blank 96 well plate and the plate is read at 340nm for a background reading. This background is performed before the addition of the ethanol reagent since other prescription medications absorb at the same wavelength, which could interfere with the assay. Ethanol reagent, which consists of NAD and ADH, is then added to the plate and the plate is read again at 340nm. The resultant reaction causes a change in absorbance, which is directly proportional to the amount of alcohol present.

Both positive and negative specimens were collected from 560 patients in a Methadone treatment clinic and tested in the ethanol microplate assay using a cutoff of 2.5mg/dL. Ethanol analysis was then performed by Headspace GC/FID (Gas Chromatography/Flame Ionization Detection) using a cutoff of 2.5mg/dL.

The enzymatic assay was tested for precision, stability of the sample, stability of the ethanol reagent and the effects of potential cross-reactants. The total precision for 5 consecutive days of testing yielded CV's less than 10%. The assay was tested and compared against the following possible cross-reacting alcohols: n-butanol, isopropanol, methanol, ethylene glycol, and acetone. The following adulterants were also tested for interference with the assay: sugar water, toothpaste, antacid, antiseptic, cola, and cranberry juice.

Of the 560 patients tested, 19 were positive by both the enzymatic assay and GC/FID. The range of positive results was 2.9 to 150mg/dL of diluted oral fluid sample. The results would need to be multiplied by three to correct for the dilution of the oral fluid in the collection device. The results yielded 99.3% agreement between the enzymatic assay and GC/FID using a cutoff of 2.5mg/dL of diluted oral fluid for both methods.

Oral Fluids, Ethanol, Intercept

K14 Postmortem Redistribution: Practical Considerations in Death Investigation

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After attending this presentation, the attendee will be able to appreciate issues of postmortem redistribution and how practical accounting for this phenomena is of value in death investigation. Use of heart and peripheral (femoral) blood findings along with background investigation and prompt case disposition facilitates interpretation of cause and manner of death. These postmortem case finding considerations allow practical insights into understanding the impact of drug redistribution in interpretation of cause and manner of death.

Postmortem redistribution (PMR) of drugs in heart blood samples has been an ongoing subject of debate in interpretation of cause and manner of death (COD, MOD). The phenomenon's origins through drug redistribution along significant concentration gradients, release from protein bound sights, movement of drug due to decomposition bacterial activity and traumatic contamination is the subject of a large number of research papers. Drug redistribution occurs to the greatest extent in the initial 24 hours following death, and refrigeration with facilitated case disposition will retard the process. Arguably, the importance of the size and extent that redistribution plays in interpreting postmortem findings in routine medical examiner cases remains a topic of debate.

The Wayne County Medical Examiner's office (WCMEO) has an annual caseload of 2,900-3,300 cases. The large majority of cases arrives at the office within 24 hours of being reported and is placed into refrigerated storage. Toxicology studies on this population have demonstrated an incidence of up to 15% of death either directly or indirectly due the presence of drugs in the general population and up to 54% in the pended case population (Table 1).

Table 1: WCMEO Drug Related Fatalities 1998-2002

YEAR	# DRUG DEATH CASES	# PENDING CASES (%) DRUG DEATH	#TOTAL CASES (%) DRUG DEATH
1998	370	692 (53.4)	2924 (12.6)
1999	404	831 (48.6)	3288 (12.2)
2000	495	916 (54.0)	3306 (14.9)
2001	470	990 (47.4)	3263 (14.4)
2002	466	1089 (42.8)	3178 (14.7)

Practical considerations of case history, time of death, time of arrival at the Medical Examiner's office, comparison to reference data, and a consistent system of collection, storage and analysis protocols, can lead to a reasoned and meaningful assessment of postmortem drug findings. Tabulation of over 5 years of analysis data of comparative heart blood and femoral blood data for 50 drug analytes are presented on Table 2. These data are presented as a function of drug Vd heart blood (B1)/ peripheral blood (B2) average ratio, median ratio, SD, and ratio range for cases of an "n" of 4 or greater. Heart bloods were collected primarily from the left side of the intact heart by needle puncture. Peripheral blood samples, 6.93 mL (+/- 4.24 mL SD) were collected from non-ligated femoral veins 96.2% of the time with the remaining samples collected from a subclavian or a non-specified "peripheral" site.

Table 2: Tabulation of Postmortem Redistribution Ratios for Heart and Femoral Blood Concentrations

DRUG	Vda L/kg	n	B1/B2b Ratio Average	B1/B2b Ratio Median	SD[c]	B1/B2[b] Ratio Range Low	B1/B2[b] Ratio Range High
Acetaminophen	0.8-1.0	16	1.083	1.037	0.345	0.693	1.987
Alprazolam	0.9-1.3	9	0.898	0.930	0.230	0.519	1.273
Amitriptyline	6-10	52	1.982	1.408	1.880	0.273	9.885
Benztropine	NAd	11	2.490	1.231	2.484	0.600	7.667
Bupropion	40	7	1.033	0.652	0.956	0.375	3.099
Carbamazepine	0.8-1.8	4	0.969	1.003	0.144	0.782	1.088
Chlorpheniramine	5.9	6	1.171	1.159	0.518	0.560	1.880
Chlorpromazine	10-35	6	1.968	0.946	2.564	0.762	7.193
Citalopram	12-16	10	2.492	1.536	2.908	0.727	10.400
Clozapine	2-7	10	1.684	0.991	2.227	0.753	8.000
Codeine	3.5	21	1.385	1.243	0.852	0.064	3.750
Cyclobenzaprine	NA	8	1.220	1.192	0.523	0.500	2.056
Desipramine	22-59	5	2.795	1.410	2.871	0.929	7.727
Desmethyldiazepam	NA	45	1.268	1.250	0.449	0.288	2.308
Dextromethorphan	255-316	11	1.721	1.694	0.856	0.105	3.158
Diazepam	0.7-2.6	38	1.299	1.235	0.533	0.331	3.053
Diltiazem	3-13	9	3.271	1.515	5.302	0.879	17.333
Diphenhydramine	3-4	48	1.956	1.536	1.487	0.356	8.200
Doxepin	9-33	22	1.604	1.258	0.939	0.612	3.300
Doxylamine	2.7	6	3.808	1.877	4.764	0.625	13.333
EDDP (Meth. Mtb.)	NA	4	0.686	0.540	0.433	0.351	1.313
Fluoxetine	20-42	21	2.395	1.379	1.917	0.586	5.909
Hydrocodone	3.3-4.7	20	1.580	1.275	0.824	0.576	3.646
Hydroxyzine	13-31	4	1.018	1.066	0.416	0.481	1.459
Meperidine	3.7-4.2	4	2.537	1.739	1.877	1.360	5.313
Mesoridazine	3-6	6	1.508	1.581	0.622	0.704	2.222
Methadone	4-5	53	1.684	1.290	1.113	0.327	5.185
Mirtazepine	10-14	4	1.064	1.125	0.612	0.269	1.737
Morphine	2-5	24	1.231	1.155	0.580	0.045	2.846
Nicotine	1.0	41	1.363	1.200	0.749	0.157	3.846
Norclozapine	NA	5	1.116	1.123	0.239	0.847	1.429
Nordoxepin	NA	20	2.720	1.967	2.204	0.280	7.400
Norfluoxetine	NA	7	2.751	1.500	2.034	1.000	5.550
Norsertraline	NA	16	2.092	1.673	1.505	0.241	5.263
Nortriptyline	20-57	43	2.152	1.235	1.896	0.196	9.600
Olanzapine	10-20	22	1.902	1.540	1.120	0.360	5.357
Orphenadrine	4.3-7.8	5	2.308	1.688	1.654	1.143	5.158
Oxycodone	1.8-3.7	4	2.196	2.203	1.031	1.200	3.167
Paroxetine	3-28	24	2.993	1.829	2.889	0.292	10.278
Phentermine	3-4	7	1.887	1.857	1.103	0.720	3.898
Promethazine	9-19	4	1.215	0.747	1.168	0.430	2.938
Propoxyphene	12-36	62	1.622	1.211	1.095	0.365	4.571
Salicylate	0.15-0.20	4	1.058	0.882	0.395	0.821	1.646
Sertraline	76	16	2.452	1.407	2.433	0.415	9.167
Thioridazine	18	5	1.277	1.111	0.713	0.600	2.130
Tramadol	2.6-2.9	9	1.358	1.431	0.465	0.556	2.107
Trazodone	0.9-1.5	14	1.535	1.182	1.095	0.583	4.800
Venlaflaxine	4-12	6	1.506	1.495	0.403	0.864	2.071
Verapamil	2.5-6.5	4	1.595	1.440	0.707	1.000	2.500
Zolpidem	0.5-0.7	8	1.474	1.452	0.337	1.056	2.000

a Vd = Volume of distribution values taken from Randall C. Baselt, "Disposition of Toxic Drugs and Chemical in Man", 6th Edition Biomedical Publications 2002. b B1 = Heart Blood Sample; B2 = Peripheral (Femoral) Blood Sample. c SD = Standard Deviation. d NA = Not Available.

These data indicate that despite size of Vd, under the conditions of case disposition for WCMEO, some drug analytes tend towards redistribution (e.g. amitriptyline, citalopram, doxylamine, paroxetine) while others do not (e.g., acetaminophen, alprazolam, hydroxyzine, mirtazepine,). When individual case tabulations are considered, in all categories PMR may or may not be a factor in cause and manner of death. Full tabulations of all cases for each analyte with COD and MOD will be presented.

Conclusions drawn from this survey indicated that in all cases where borderline drug toxicity is an issue in COD and MOD of death, it is essential to account for PMR. These data also suggest that when actual concentrations are excessively high or low, regardless of PMR, the interpretation of drug involvement or lack thereof in COD and MOD does not change.

Postmortem Redistribution, Postmortem Release, Multi-site Testing

K15 Postmortem Production of Ethanol in Different Tissues Under Controlled Experimental Conditions

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After attending this presentation, attendees would be able to establish the level of postmortem ethanol (produced after death) under controlled experimental conditions within different time intervals and under different temperature.

The authors would like to see the results from this survey considered as a basis for further investigations with crucial aim that is very important in forensic practice - to distinguish postmortem (endogenous) production of ethanol versus ethanol ingestion before death (exogenous one).

There is the assumption that postmortem production of ethanol is in accordance with temperature increase, duration of time interval, and amount of carbohydrates in the tissue.

All the activities of this survey were performed at the Department of Forensic Medicine, Clinical Center Novi Sad, on the corpses of persons of both sexes, aged between 20 and 50 years, whose death occurred 6-12 hours before autopsy, i.e., taking the specimen. The death of the persons whose corpses were used for the analyses was of natural or violent origin and it excluded medical interventions (treatment and death in the hospital, or other medical institution), and the violent deaths caused by toxic substances. The specimen of blood, liver, skeletal muscle and kidney were taken from 30 corpses and were divided into 2 control and 3 experimental groups. The first control group of specimen was analyzed immediately after taking, and the second control group of specimen was stored at the temperature of – 20 ºC. The first experimental group of specimen was stored at the temperature of + 4 ºC, the second at +20 ºC, and the third one at +30 ºC. All experimental groups were divided into four subgroups, according to the duration of incubation at given temperature: the first subgroup was stored at appropriate temperature for 24 hours, the second for 48 hours, the third for 96 hours and fourth one, for 192 hours. Chemical ethanol analysis of the taken specimen was performed by standard gas-chromatography method.

The results show that all of the control specimen stored at -20 ºC do not show any change in ethanol quantity, in all time intervals. There is no statistical significance of ethanol quantity change remarked in any tissue stored at +4 ºC at any time interval. At the temperature of +20 ºC, all tissues, except blood, show statistically significant ethanol quantity change referring to time intervals, comparing with controls. The postmortem production of ethanol at +30 ºC is increased due to the course of time, in all tissues. Statistically significant ethanol quantity change appears on the 1st day (kidney, muscle and liver tissue) and 2nd day (blood) at +30 ºC, while at +20 ºC it appears predominantly on the 2nd day (kidney, liver and muscle tissue). Significant increase of produced ethanol in liver, kidney and muscle tissue at +30 ºC is noted up to particular time interval (liver – 4th, kidney and blood – 2nd, muscle 1st day), after which these levels are mildly decreased without statistical significance, except in blood tissue, where the significant decrease was found. The absolute range of produced ethanol reaches the highest level in liver tissue.

On the basis of the results gained during this survey, we can confirm the assumptions as follows: 1. the postmortem production of ethanol occurs and it varies in different tissues; 2. postmortem production of ethanol is increased by rise in temperature; 3. postmortem production of ethanol depends on the tissue amount of carbohydrates (liver – glycogen); 4. postmortem production of ethanol is increased, in general, in accordance with the course of time. It is observed, too, that postmortem production of ethanol is increased up to particular time interval at +30 ºC, after which the values of measured ethanol are mildly decreased.

Postmortem Production, Ethanol, Experimental Conditions

K16 The Measurement of Uncertainty for Toluene Analysis in Biological Fluids by HS-GC

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After attending this presentation, attendees will understand the uncertainty estimation process and to validate the method in forensic field.

Toluene has been widely used as an industrial solvent. Sniffing of thinners or adhesives containing toluene, which is illegal in Korea, is known to occur. The determination of toluene level in biological fluids such as blood and urine is a powerful tool for monitoring toluene exposure and for evaluation of toluene inhalation. The aim of this work was to validate the method of toluene determination and obtain the uncertainty estimate around cut-off level. The chromatographic conditions of the method employ an HP INNOwax capillary column (30m x 0.25mm, film thickness 0.25um), programmed condition (60 ºC(6min), 10 ºC/min, 140 ºC(3min)) with He at a column flow of 1.0ml/min, injector and detector temperature at 240 ºC, a split ratio of 30:1. Sealed sample vials containing biological sample 1ml, buffer 2ml and iso-butanol 50ul as an internal standard were heated at 60 ºC for 20 minutes in headspace autosampler and injected into GC with FID. The linearity of the toluene peak area responses was demonstrated from 0.05ppm to 100ppm. Repeatability and reproducibility of the toluene peak area responses showed R.S.D. of 3.6% and 4.6 %, respectively. The limits of detection and quantitation were determined to be 0.01ug/mL and 0.02ug/mL in water and 0.02ug/mL and 0.05ug/mL in urine, respectively. The other parameters such as selectivity, sensitivity, accuracy and recovery were also examined. The measurement uncertainty for toluene analysis was estimated from experimental results. We determined 0.068ug/mL as the uncertainty for cut-off level, 0.1ug/mL.

Toluene, Uncertainty, Validation

K17 Comparison of Calibration Approaches for the Quantitative GC/MS Analysis on Secobarbital

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After attending this presentation, attendees will be familiar with the characteristics of the calibration curves resulting from the use of isotopic analogs of the analyte as the internal standards (ISs). Specific parameters studied include (a) ion cross-contribution and (b) column temperature programming conditions that may affect the use of calibration approaches.

This study was placed on practically evaluating the calibration approach by using ^{2}H- and ^{13}C-analogs as ISs for the quantitative determination by GC/MS in urine. An automatic well-established solid-phase extraction and methylation procedures were used prior to the GC/MS measurement. The cross-contribution of ions designated for the analyte and its IS were evaluated by the "direct normalized measurement" method through selected ion monitoring (SIM) mode. The spiked IS magnitude and reconstitute volume were also evaluated for the appro-

priate GC/MS determination at low concentration level. To decrease the cross-contribution, 50 ng/mL $^2H_5$-analog and 25 ng/mL $^{13}C_4$-analog ISs were respectively added into each standard solution. One-point, linear, hyperbolic and polynomial calibration approaches were used to investigate the quantitative effectiveness based on the comparison of theoretical and observed concentrations of standard solutions containing 10 to 800 ng/mL secobarbital. Two GC column temperature programming conditions, 20 ºC high ramp rate and 2 ºC low ramp rate, were adopted to generate different degrees of peak-overlap and ion cross-contribution for the purpose of evaluating the most appropriate application for each calibration approach.

Ion cross-contribution and the "over-all non-proportional change in ionization efficiency" phenomenon have been regarded as the underlying causes to change the theoretical analyte/IS ratios. Data shown in Table 1 indicate that cross-contribution deriving from IS to the analyte leads to the positive observed concentration at low concentration levels by one-point calibration. This phenomenon obviously shows that 13 % m/z 195 ion contributed from IS generates higher observed concentration values at low concentration levels than that of 1.9 % m/z 196 ion. Thus, the ion-pair with the less amount of ion cross-contribution should be the most appropriate candidate for the quantitation by using the one-point calibration approach. This trend also reveals that the more intensity of ion-pair ratios resulting from ion cross-contribution increases, the more peak-overlapping under 20 ºC high ramp rate does as well. The ratios at low concentration levels become farther to the "expected" values based on the ion cross-contribution resulting from the IS. Temperature programming is the other interference factor in the quantitative determination by one-point calibration. To determine the lower concentration levels, the lower ramp rate is a better temperature programming. The deviation obtained in comparison the theoretical with the observed concentration in standard solutions at low concentration levels by linear calibration was obviously lower than that by one-point approach. Some figures even reduce to negatives. This trend presents that ion cross-contribution generating higher ion-pair ratios at low concentration levels can be adjusted based on the lower ion-pair ratios deriving from the "non-proportional over-all change in ionization efficiency" phenomenon at high concentration levels. Thus, the linearity of the calibration curve increases, especially at low concentration range. Due to the slight increase of ion cross-contribution along with the increasing peak-overlap, the temperature programming with high ramp rate will also bring about the higher observed concentration by linear calibration at low concentration levels Quantitation results using hyperbolic calibration show the different phenomenon. The ion-pair with the higher ion cross-contribution leads to the lower observed concentration and deviation. This trend indicates that the characteristic of the hyperbolic curve is suitable for standard solutions with the higher ion cross-contribution. Thus, the quantitative effectiveness for low concentration levels using high ramp rate are better than those using low ramp rate on GC temperature program. Resulting data using polynomial calibration demonstrate that all of ion-pairs generate ideal quantitation without interference caused by ion cross-contribution and GC temperature programming. Polynomial curves can appropriately fit in ion-pair ratio of each standard solution. The only defect is the complicated procedure used to solve the equations obtaining from polynomial regression.

Ion cross-contribution is the underlying cause to interfere with the quantitative determination by one-point and linear approaches at low concentration range. This situation can be improved by GC temperature programming. On the contrary, hyperbolic calibration can be used for the ion-pair containing high ion cross- contribution. Polynomial calibration is an ideal approach because there is no need to select an ion-pair via the time-consuming evaluation.

Table 1. Comparison of quantitation results using different ion pairs and calibration approaches—
Analyte/IS: Secobarbital/$^2H_5$-analog.

Ion-pair§ m/z	Temp. Program	Theor. Conc. ng/mL	Ion Ratio	Obs'ed conc. Dev.% by One Point	Obs'ed conc. Dev.% by Linear	Obs'ed conc. Dev.% by Hyperbolic	Obs'ed conc. Dev.% by Polynomial
196/201	2 ºC ramp rate	10	0.1909	9.843(-1.6)	9.610(-3.9)	7.654(-23.5)	9.859(-1.4)
		20	0.4016	20.71(3.5)	20.36(1.8)	18.74(-6.3)	20.12(0.6)
		50	0.9697	Calibrator	49.35(-1.3)	48.59(-2.8)	48.09(-3.8)
		100	2.070	106.8(6.8)	105.5(5.5)	106.2(6.2)	103.4(3.4)
		200	3.895	200.8(0.4)	198.6(-0.7)	201.2(0.6)	197.9(-1.1)
		400	7.702	397.1(-0.7)	392.8(-1.8)	397.1(-0.7)	400.6(0.1)
		800	15.73	810.9(1.4)	802.3(0.3)	800.5(0.1)	797.8(-0.3)
	20 ºC ramp rate	10	0.2206	11.16(11.6)	10.57(5.7)	9.232(-7.7)	10.44(4.4)
		20	0.4036	20.42(2.1)	20.06(0.3)	18.92(-5.4)	19.72(-1.4)
		50	0.9884	Calibrator	50.36(0.7)	49.83(-0.3)	49.56(-0.9)
		100	1.974	99.84(-0.2)	101.4(1.4)	101.8(1.8)	100.4(0.4)
		200	3.877	196.1(-1.9)	200.0(0.0)	201.8(0.9)	199.9(-0.0)
		400	7.645	386.7(-3.3)	395.2(-1.2)	398.1(-0.5)	399.9(-0.0)
		800	15.49	783.7(-2.0)	801.8(0.2)	800.3(0.0)	801.0(0.1)
195/200	2 ºC ramp rate	10	0.4298	13.18(31.8)	8.568(-14.3)	7.971(-20.3)	9.968(-0.3)
		20	0.7075	21.70(8.5)	18.01(-9.9)	17.50(-12.5)	18.86(-5.7)
		50	1.631	Calibrator	49.41(-1.2)	49.17(-1.7)	48.72(-2.6)
		100	3.347	102.6(2.6)	107.8(7.8)	108.0(8.0)	105.4(5.4)
		200	6.010	184.3(-7.8)	198.4(-0.8)	199.1(-0.4)	196.2(-1.9)
		400	11.84	362.9(-9.3)	396.5(-0.9)	397.7(-0.6)	401.8(0.5)
		800	23.73	727.8(-9.0)	801.2(0.1)	800.5(0.1)	802.4(0.3)
	20 ºCramp rate	10	0.7008	19.68(96.8)	12.28(22.8)	10.72(7.2)	11.40(14.0)
		20	0.9275	26.05(30.2)	20.18(0.9)	18.84(-5.8)	19.04(-4.8)
		50	1.780	Calibrator	49.90(-0.2)	49.32(-1.4)	47.98(-4.0)
		100	3.346	93.98(-6.0)	104.5(4.5)	105.1(5.1)	102.0(2.0)
		200	6.084	170.9(-14.6)	199.8(-0.1)	202.2(1.1)	198.8(-0.6)
		400	11.62	326.4(-18.4)	392.8(-1.8)	396.8(-0.8)	399.0(-0.3)
		800	23.35	655.7(-18.0)	801.3(0.2)	800.6(0.1)	798.9(-0.1)

§Ion cross-contribution–m/z: 196/201 (1.9 % contributed by IS; 0.33 % contributed by analyte); m/z 195/200 (13 % contributed by IS; 0.59 % contributed by analyte).

*Regression equations—For m/z 196/201: $y=0.0196x + 0.0025$ (r^2=0.9998) under 2 ºC ramp rate, $y = 0.0193x + 0.0165$ (r^2=0.9999) under 20 ºC ramp rate; for m/z 195/200: $y = 0.0294x + 0.1779$ (r^2=0.9998) under 2 ºC ramp rate, $y = 0.0286x + 0.3719$ (r^2=0.9999) under 20 ºC ramp rate.

†Regression equations—For m/z 196/201: $y = (2.3995 + x)/(-0.0020x + 52.68)$ (r^2=0.9999) under 2 ºC ramp rate, $y = (2.4480 + x)/(-0.0014x + 52.97)$ (r^2=1.0000) under 20 ºC ramp rate; for m/z 195/ 200: $y = (6.7870 + x)/(-0.0004x + 34.34)$ (r^2=0.9998) under 2 ºC ramp rate, $y = (14.383 + x)/(-0.0012x + 35.84)$ (r^2=1.0000) under 20 ºC ramp rate.

‡Regression equations—For m/z 196/201: $y = 6\times10^{-9}X^3-6\times10^{-6}X^2+0.0207X-0.0126$ (r^2=1.0000) under 2 ºC ramp rate, $y = 3\times10^{-9}X^3-3\times10^{-6}X^2+0.0198X-0.0142$ (r^2=1.0000) under 20 ºC ramp rate; for m/z 195/200: $y = 8\times10^{-9}X^3-9\times10^{-6}X^2+0.0315X-0.1167$ (r^2=0.9999) under 2 ºC ramp rate, $y = 7\times10^{-9}X^3-7\times10^{-6}X^2+0.0299X-0.3608$ (r^2=1.0000) under 20 ºC ramp rate.

Calibration, GC/MS Analysis, Isotopic Analogs

K18 The Effects of pH on the Oxidation of Ephedrine and Phenylpropanolamine Using Sodium Periodate

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The attendees will better understand the impact that pH may have on the oxidation of ephedrine and phenylpropanolamine using sodium periodate.

The use of sodium periodate to chemically oxidize common over the counter amphetamine like substances such as ephedrine, and phenylpropanolamine has become an accepted practice in forensic urine drug testing environments. However, very little information is available as to the effect that pH has on the efficiency of this oxidative procedure. The purpose of this study was to evaluate the potential of the production of amphetamine and methamphetamine in the presence of high concentrations (3,000,000 ng/ml) of ephedrine and phenylpropanolamine. A saturated sodium periodate solution and sodium hydroxide solution are added to the urine sample containing the drug and deuterated internal standards. The pH of this oxidation step is between 11 and 13. In this study, the sodium periodate was also adjusted to pH 4.4, 5.2, 9.1 and 9.3. Samples were extracted using solid phase technology, and derivatized with MBTFA to form the TFA derivative. GC/MS analysis was conducted using electron impact ionization using three ions for the native compound and two ions for the deuterated internal standard. A single point calibrator at 500 ng/ml was used to establish both qualitative and quantitative results. No amphetamine or methamphetamine was detected at any of the pH levels evaluated. This data suggests that the oxidation of ephedrine and phenylpropanolamine at levels as high as 3,000,000 ng/ml by sodium periodate is effective when the pH is between 4.4 and 13.

pH, Ephedrine, Phenylpropanolamine

K19 The Validity of Surrogate Reporting of Substance Use

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After attending this presentation, attendees will have an understanding of evaluating the validity of surrogate reporting of substance use for the deceased.This study contribute to the understanding of factors involved in achieving high sensitivity and specificity of certain substances when contesting the validity of next-of-kin reporting versus toxicology reports.

Introduction: Collection of substance use information for those who died unexpectedly often rely on proxy respondents or toxicology reports. Past research have examined surrogate reporting about the deceased characteristics. However, the direct assessment of proxy reporting of substance use versus toxicology report were rarely investigated. The purpose of this study were: 1) to use toxicology report as a gold standard to evaluate the validity of proxy reporting of substance use, and 2) to identify which drug groups that are more likely to be accurately reported by the surrogate.

Methods: The data for this study were obtained from the 1993 National Mortality Followback Survey (NMFS) conducted by the National Center for Health Statistics, Centers for Disease Control and Prevention. With the permission from next-of-kin of the deceased, questionnaire data were linked to 3483 toxicology report collected from 1265 medical examiner/coroner offices. Ten items that asking the deceased substance use behavior were selected from questionnaire and compared with toxicology report. In the interview questionnaire, substances were grouped into nine drug categories : alcohol, pain killer, sedative, tranquilizer, antidepressant, stimulant, cocaine, marijuana, and methadone. Sensitivity and specificity test were used to evaluate the validity of next-of-kin reporting. We defined sensitivity is the toxicology report GC/MS confirm positive of a substance used and next-of-kin also reported yes to that substance for the deceased. Specificity is the toxicology report GC/MS confirm negative of a substance used and next-of-kin also reported no to that substance for the deceased.

Results and Discussion: The study results in the table below demonstrated that methadone and painkillers such as morphine, codeine and propoxyphene had 100% sensitivity. High sensitivity reflects that immunoassay procedures and confirmation techniques for these two categories of substance that were well developed and routinely executed for their identification in the laboratory. However, the sensitivity for other categories of substance use was low. The possible explanation for low sensitivity could be: 1) inability of the laboratory to detect substances that were not routinely screened and confirmed; 2) each substance has an unique halflife.

Consumption of substances such as methamphetamine, cocaine, or alcohol few days prior to death often provides a negative lab result; 3) small quantity of substance use frequently causes a lab result below the detection limit; 4) detection time also varies depending upon analytical method used, drug metabolism, individual's physical condition, fluid intake, and method and frequency of substance ingestion prior to death. Furthermore, cutoff values for positive substance vary from one laboratory to another. Regarding specificity, the survey revealed an average 76% specificity indicating that proxy reporting of substance use has some degree of scientific certainty in general. However none of the individual categories of substance reached 100% specificity, meaning proxy respondents did not know whether the decedents had used substance prior to their death. Alcohol, on the other hand, has the lowest specificity. It is probably associated with proxy's social, financial, psychological and legal implications. In conclusion, our study showed that both the toxicology report and proxy reporting provided important information in identifying forensic relevance for those who died unexpectedly. Nevertheless, shortfalls of each reporting system should be cautiously taken into consideration in result interpretation.

	Sensitivity	Specificity
Alcohol	0.93	0.26
Pain Killer	1.00	0.73
Sedative	0.36	0.88
Tranquilizer	0.47	0.85
Antidepressant	0.60	0.75
Stimulant	0.41	0.93
Cocaine	0.59	0.85
Marijuana	0.58	0.80
Methadone	1.00	0.90

Validity, Toxicology Report, Surrogate Reporting

K20 Death Attributed to Quetiapine Overdose

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This is a case report where the cause of death was attributed to quetiapine overdose. There is little literature regarding minimum lethal concentrations of this drug, and presentation of this case may help with compiling such data.

Quetiapine (Seroquel) is an antipsychotic drug belonging to a new chemical class, the benzothiazepine derivatives. We present the first case of quetiapine overdose causing death reported in the Province of British Columbia.

A 56-year-old Caucasian female was found unresponsive on the bedroom floor. The deceased's medical history included bipolar disorder and severe obsessive-compulsive disorder. A full autopsy was performed approximately 32 hours after death. Significant findings included evidence of localizing interstitial pneumonitis. There was no evidence of underlying chronic lung disease or of an aspiration event. Specimens were collected for toxicological analysis.

The blood specimen was initially subjected to a thorough qualitative analysis. Basic drugs were screened for by liquid-liquid extraction followed by GC-NPD and GC-MS electron impact detection. Acidic and neutral drugs were screened for by liquid-liquid extraction followed by HPLC-DAD. Volatiles were assayed by GC-FID. Qualitative analysis identified acetaminophen, carbamazepine, lorazepam, clonazepam, diphenhydramine and quetiapine. The concentration of acetaminophen was less than 10 mg/L, carbamazepine was 8.5 mg/L (36 umol/L), lorazepam was 0.05 mg/L (0.16 umol/L), and clonazepam was 0.027 mg/L (0.086 umol/L). With the exception of acetaminophen, which is less than therapeutic, these concentrations are consistent with levels achieved therapeutically. The concentration of diphenhydramine was 3.7 mg/L (14 umol/L); although this is greater than the therapeutic range (0.010 – 0.10 mg/L) it is less than the commonly accepted minimum lethal level of 8 mg/L.

Quetiapine was assayed in biological specimens as follows: briefly, to each tube add 1 mL of appropriate fluid, 50 uL of Internal Standard (Hydroxytriazolam 0.01 mg/mL), 1 mL of saturated sodium carbonate solution was added, and extracted into 6 mL n-butyl chloride. The extract was concentrated under nitrogen, reconstituted and derivatized with 50 uL of MTBSTFA, heated at 60°C for 30min, and 1 uL was injected into an Agilent model 5890 gas chromatograph coupled to a NP Detector using a 12 m Ultra-1 (0.33um film thickness) capillary column (Agilent). The initial temperature was 260 ^{o}C and increased 10 ^{o}C/min for one min then 50 ^{o}C/min until 300 ^{o}C, then held for 2 min. The concentration was measured by comparison of peak height ratios of quetiapine to that of hydroxytriazolam against a standard curve. Linearity was observed up to 2.0 mg/L. Samples with concentrations exceeding the linearity were diluted.

Elevated concentrations of quetiapine were found in blood 7.20 mg/L (19 umol/L) and in vitreous fluid 0.93 mg/L (2.4 umol/L). Quetiapine is well absorbed from the gastrointestinal tract and reaches peak plasma levels 1.5 hours after oral administration. The drug's half-life ranges from 2.7-9.3 hours. The volume of distribution of quetiapine is 10 L/kg and it is 83% bound to plasma proteins. The specimen in this case were approximately 7 fold greater than the reported therapeutic range (0.1 – 1.0 mg/L), assuming the red cell serum distribution ratio is 1:1, and is comparable to that reported in the literature to be associated with serious/potentially fatal toxicity. The cause of death was ascribed to solely quetiapine overdose.

Quetiapine, Overdose, Fatal

K21 Determination of Clonidine in Postmortem Specimens by LC/MS/MS

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The attendee of this presentation will be introduced to the procedural details of a LC/MS/MS methodology for measurement of clonidine in postmortem specimens. In addition, postmortem clonidine concentration data from a series of cases will be presented and the significance of these findings will be discussed.

The impact of this presentation includes an improved analytical approach for the detection of clonidine in postmortem specimens and documentation of additional postmortem clonidine concentration data to the forensic toxicology community to better facilitate interpretation.

Clonidine Hydrochloride (Catapres, Clorpres, Combipres, Duraclon), an imidazoline derivative synthesized in the early 1960s, is primarily prescribed as an antihypertensive agent available in both oral form and as a transdermal patch. Clonidine acts as an alpha2-adrenergic receptor agonist resulting in reduced sympathetic outflow from the central nervous system, thus leading to a reduction in blood pressure. Recently clonidine has been indicated as continuous epidural infusion for treatment of severe pain in cancer patients that is not relieved by opiate analgesics alone. Other uses for clonidine include treatment of anxiety and attention-deficit hyperactivity disorder (ADHD), as a preoperative sedative, and treatment of withdrawal from narcotics and nicotine. Oral dosages of clonidine hydrochloride are available in 0.1 mg, 0.2 mg, and 0.3 mg tablets with typical daily doses from 0.2 to 0.6 mg/day. Therapeutic plasma concentrations vary between 0.7 and 3.8 ng/mL.

The primary adverse effects of clonidine are dry mouth and sedation. Due to its hypnotic effect, clonidine has been used to incapacitate and subsequently rob victims, and suggested as an effective agent in drug facilitated sexual assault. Blood clonidine concentrations in the reported cases of chemical submission ranged from 13 – 68 ng/mL. Clonidine may also impair driving especially when taken with alcohol and other sedative drugs. Symptoms of overdose include early hypertension followed by hypotension, bradycardia, respiratory depression, and hypothermia. Children are reported to be especially susceptible to the sedative effects of clonidine. A serum concentration of 3.5 ng/mL produced unconsciousness in one child. After intensive treatment, a 28-year-old male survived a 100 mg overdose of clonidine and exhibited a peak plasma concentration of 370 ng/mL.

Clonidine is not detected in routine toxicological screening methods used in most laboratories conducting postmortem analysis. The low therapeutic and toxic concentrations encountered in blood coupled to the need for derivatization prior to GC/MS analysis contribute to this fact. The aim of this work is to develop a simple and sensitive LC/MS/MS analytical procedure for detection of underivatized clonidine and document the occurrence of clonidine in postmortem specimens from casework conducted in our laboratory. Cases for this study were selected based upon clonidine appearing in the list of medications collected at the scene.

Clonidine and 7-aminoflunitrazepam, as the internal standard, were isolated from alkaline postmortem fluid and tissue homogenate by extraction with n-butyl chloride. The n-butyl chloride fraction was evaporated to dryness. The residue was reconstituted in mobile phase, washed with hexane (saturated with mobile phase) and submitted for analysis by LC/MS/MS. Liquid chromatography was performed using an Agilent 1100 with Agilent Zorbax Eclipse XDB-C8 column. The column was 150mm by 4.6mm with 5 micron film. The mobile phase was an isocratic mixture of methanol (55%) and water (25 mmol ammonium formate pH 3.0) (45%). The LC flow (0.25 ml/min) was

directed into an electrospray ionization source and mass spectral analysis was performed with an API 2000 triple quadrupole mass spectrometer (Applied Biosystems).

Qualitative identification of clonidine was established by monitoring two transition ion pairs, m/z 229.9/212.8, m/z 229.9/171.8. Quantitative analysis monitored the peak areas of the m/z 229.9/212.8 transition for clonidine and the 284.1/135.0 transition for 7-aminoflunitrazepam. A five point curve from extracted blood calibrators, ranging from 0.4 to 4.0 ng/mL, was generated, r2 =0.9988. The established limit of detection was 0.1 ng/mL. Of nine cases screened thus far, postmortem blood clonidine concentrations ranged from 0.64 – 6.9 ng/mL.

Clonidine, LC/MS, Postmortem

K22 Death Due to Ingestion of Tramadol in London, UK

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Attendance at this presentation will enable the participant to study an unusual toxicological case in London involving tramadol. The presentation will also enable the participant to learn how such cases are processed by the Forensic Toxicology Service in London, UK.

This presentation is important to the toxicological and analytical community as it is the first such case in London, UK involving the highest reported level of tramadol in addition to a concentration of enalapril consistent with therapy. Considering the lack of any other significant autopsy findings, the results of our toxicological analyses are consistent with the assumption of a fatal overdose of tramadol producing a high concentration of the drug, exceeding any noted in the UK before.

The Forensic Toxicology Service offers a screening and quantification toxicology service to most of Her Majesty's Coroners and Forensic Pathologists in London as well as various Police Forces and one branch of the Armed Forces. As a result, we are required to screen for a large number of prescribed and illicit drugs in post-mortem specimens followed by quantification of those detected. All analyses must be completed and our final report must be submitted to the Courts within 15 business days of the arrival of the case at the Service. This case was presented to the Service in May 2003 and involved a 79-year-old Caucasian female with a history of pain related health problems, which required prescription of various analgesics, including tramadol. At the home of the deceased officers discovered empty boxes of tramadol and zopiclone as well as boxes of co-codamol (preparation of codeine and paracetamol), Duragesic® patches (fentanyl) and paracetamol. We were requested to subject the unpreserved post-mortem blood and urine specimens of the deceased to our standard alcohol and general drug screen in order to facilitate HM Coroner in his Inquest into this death.

The case blood and urine specimens were screened for alcohol and determined negative. Similarly, paracetamol (i.e., acetaminophen) and salicylates were not detected. Our benzodiazepine screen by HPLC-MS-MS on the blood specimen did not detect any benzodiazepines or metabolites. Enalapril was detected by HPLC-MS-MS and, when quantified in blood, it measured 0.02 mg/L, which fell well within low levels observed in therapy. Using our standard liquid-liquid drug extraction scheme for basic (i.e. alkaline) drugs followed by gas chromatography – mass spectrometry (GC-MS), we were able to identify tramadol in both the blood and urine case specimens. The urine specimen also showed a spot under UV illumination following thin layer chromatography. When the TLC plate was sprayed with FPN reagent (ferric chloride; perchloric acid; nitric acid), the spot showed a strong purple color. When quantified by GC-MS using appropriate calibrators and controls, tramadol measured 10.3 mg/L in blood. Urine tramadol was not quantified.

Tramadol is prescribed in the United Kingdom as an opioid analgesic to treat moderate to severe pain as non-proprietary 50-mg capsules or 50mg/mL injection, or under various trade names including Zamadol® and Zydol® capsules and Dromadol® and Zydol® XL modified release tablets. Enalapril is prescribed in the UK to treat hypertension and also to alleviate symptomatic heart failure. It is available as non-proprietary tablets (2.5, 5, 10 or 20 mg) as well as under the trade names of Innovace® and Innozide®.

After reviewing the scientific literature on tramadol related fatalities, it was noted that published post-mortem blood levels in such cases ranged from 3.7mg/L (Loughrey et. al., 2003) to 9.6mg/L (Musshoff and Madea, 2001). Tramadol-related deaths and non-fatal intoxications have previously been studied and tramadol levels ranged from 0.03 to 22.59 mg/L (Goeringer et. al., 1997; Levine et. al., 1997). Our case is important to the toxicological and analytical community as it is the first such case in London, UK involving the highest reported level of tramadol in addition to a concentration of enalapril consistent with therapy. Considering the lack of any other significant autopsy findings, the results of our toxicological analyses are consistent with the assumption of a fatal overdose of tramadol producing a high concentration of the drug, exceeding any noted in the UK before.

Tramadol, GC?MS, Fatality

K23 Investigation of the Analytical Degradation of Clozapine-n-Oxide to Clozapine in a Postmortem Case

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Attendees should be able to use this information to assist with the interpretation of cases involving clozapine.

The goal of this presentation is to provide forensic toxicologists with data from a recent postmortem case in which clozapine was determined to be the cause of death. While clozapine-n-oxide does convert to clozapine with analysis by gas chromatography, our results indicate that this may be an insignificant phenomenon.

Clozapine is a tricyclic dibenzodiazepine used for the treatment of severe schizophrenia patients that have failed to respond to more standard therapy. Clozapine undergoes extensive metabolism including N-demethylation, N-oxidation, chlorine ring oxidation, chlorine and thiomethly conjugation. Clozapine-n-oxide is a major metabolite found in plasma with little pharmacological activity.

A 40-year-old male suffering from schizophrenia became agitated and self destructive in the assisted living facility where he was a resident. He was taken to the emergency room where he expired after extensive resuscitative efforts. Autopsy results were negative. Blood, vitreous, liver and brain specimens were submitted to the toxicology laboratory for analysis. Routine drug and alcohol screens using gas chromatography and gas chromatography/mass spectrometry were positive for clozapine, bupropion, chlorpheniramine, atropine, and desmethylsertraline. Clozapine was quantitated with gas chromatography with nitrogen phosphorous detection (GC/NPD), gas chromatography with flame ionization detection (GC/FID) and high performance liquid chromatography (HPLC).

For the GC analyses, 1.0 mL blood or 1.0 g of a 1:4 liver homogenate spiked with 2.0 mcg olanzapine (internal standard) were extracted with 7.5 mL 1-chlorobutane following alkalinization with 0.5 mL concentrated ammonium hydroxide. The organic layer was transferred and back extracted into 2.5-mL sulfuric acid. The solvent layer was removed. The aqueous acid layer was made alkaline with 0.5 mL concentrated ammonium hydroxide and extracted with 3.0-mL chloroform. Following centrifugation, the aqueous was removed and the chloroform layer was dried to residue under nitrogen at 40°C. The

samples were reconstituted with 50-mcL methanol and 2.0 mcL was injected on an HP 5890 gas chromatograph with nitrogen-phosphorous detection or an HP 6890 gas chromatograph with flame ionization detection. The assay is linear from 0.5 mcg/mL to 8.0 mcg/mL with a limit of detection of 0.5 mcg/mL clozapine.

For HPLC analyses, 1.0 mL blood or 1.0 gram of a 1:4 liver homogenate spiked with 2.0 mcg promazine (internal standard) was extracted with 7.5 mL 3% isopropanol in pentane following alkalinization with carbonate buffer (pH 9.5). The extracts were dried to residue under nitrogen at 40°C. The samples were reconstituted with 200 mcL acetonitrile, filtered with a 0.2 um syringe filter and 20 mcL were injected on the Varian 5500 HPLC equipped with a cyanopropyl column and ultraviolet detection. The samples were analyzed using ultraviolet detection at 257 nm and a flow rate of 1.5 mL/min for the 60:35:5 acetonitrile: ammonium acetate: methanol mobile phase.

Clozapine-n-oxide has been shown to convert to clozapine by thermal degradation by GC analysis, in vivo processes in animals, and base reduction during extraction. We investigated the influence of clozapine-n-oxide on the results obtained by gas chromatography for the present case. Injection of unextracted clozapine-n-oxide on GC/NPD resulted in the detection of clozapine plus other unknown products. Three clozapine and 3 clozapine-n-oxide blood controls (2.0 mcg/mL, each) were spiked and extracted using the alkaline liquid-liquid method above. They were compared to extracted standard curves for each compound ranging from 0.5 to 8.0 mcg/mL. The mean concentration of clozapine (± SD) from the clozapine controls was found to be 2.2 (± 0.1) mcg/mL. The mean concentration of clozapine found when the extracted clozapine-n-oxide samples were compared to the clozapine curve was 1.1 (± 0.07) mcg/mL. When the clozapine-n-oxide extracts were compared to the clozapine-n-oxide standard curve, a mean of 1.8 (± 0.1) mcg/mL was obtained.

Two experiments were performed using HPLC. First, standards were prepared and subjected to the extraction protocol outlined above (blank, negative control, 0.5 mcg clozapine, 0.5 mcg clozapine-n-oxide, 0.5 clozapine + 0.5 mcg clozapine-n-oxide). We found no conversion of clozapine-n-oxide to clozapine. Secondly, a clozapine standard curve (0.5 to 8.0 mcg/mL blood) was extracted and used for comparison to 2 blood controls, 1 containing 1.0 mcg/mL clozapine-n-oxide, the other 1.0 clozapine-n-oxide + 2.0 mcg/mL clozapine. The control containing only clozapine-n-oxide demonstrated no clozapine conversion. The control containing both compounds showed the expected 2.0 mcg/mL clozapine.

These results indicate that clozapine-n-oxide does convert to clozapine during GC analysis and/or under strong alkaline conditions. Our HPLC analysis showed no conversion of clozapine-n-oxide to clozapine. Interestingly, when the blood from the postmortem case was extracted and analyzed by both the GC and HPLC protocols, the same result was obtained: GC, 2.1 mcg/mL; HPLC, 2.0 mcg/mL. These results indicate that the likely presence of clozapine-n-oxide did not cause significant overestimation of clozapine in a long-term user of this drug.

Clozapine, Clozapine-N-Oxide, Chromatography

K24 Demographic and Toxicological Profiles of 127 Decedents Testing Positive for Ephedrine Alkaloids

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Attendants will learn the basic profile of ephedrine-related deaths, as well as the relationship between blood ephedrine, blood norephedrine concentrations, and possible episodes of ephedrine toxicity.

This presentation will impact the forensic community by providing the substantial toxicological and pathological data needed to make informed diagnostic decisions about cause of death when ephedrine is detected by postmortem toxicological screening.

The relative safety of ephedra-containing dietary supplements is disputed, and the toxicology of ephedra-supplements remains poorly understood. Two theories have been advanced to account for purported episodes of ephedrine toxicity; (1) It has been suggested that humans may metabolize ephedrine to form norephedrine (phenylpropanolamine) which, in turn, may cause dangerous elevations of pulse and blood pressure; (2) It has been suggested that, when ephedrine-related toxicity occurs, it "results from accidental overdose often prompted by exaggerated off-label claims and a belief that 'natural' medicinal agents are inherently safe. " According to this theory ephedrine toxicity results from inadvertent overdosage, occurring when consumers ingested poorly produced supplements containing unpredictable amounts of ephedrine. Both theories are plausible, but evidence is lacking. Accordingly, a review of all autopsies performed in our Medical Examiner's jurisdiction was undertaken, from 1994 through 2000, where ephedrine or ephedrine-related compounds (E+) were detected in blood or urine drug screening. Methods: When available, urine samples were initially screened with the polyclonal EMIT test. When no urine was available, blood was screened using GC/MS. Following alkaline extraction, ephedrine, pseudoephedrine, and norephedrine were identified by gas chromatography with nitrogen-phosphorus detection. Samples were subsequently confirmed using full scan electron impact mass spectrometry. Other drugs were identified following a similar protocol. Drug concentration values in E+ cases, where trauma was the cause of death, were compared with values in E+ cases dying of all other causes. Results: A total of 127 cases were identified. The mean postmortem interval was 17.4 hours in the trauma group, and 18.4 hours in the non-trauma groups (not significantly different). The mean age for the 127 cases was 44.9 ± 1.2 years. Fifty-nine percent were Caucasian, 22.8% black, 10.2% Asian, and 3.9% Hispanic. Decedents were mostly male (80.3%). Thirty-three (25.9%) died of trauma. Mean blood ephedrine concentrations in trauma vs. non-trauma were not significantly different (1.27 mg/L, SD = 2.49 for trauma cases vs. 1.61 mg/L, SD = 2.47 for non-trauma cases, $p = 0.603$). Blood ephedrine concentrations were < 0.49 mg/L in 50% of the cases, and ranged from 0.07 to 11.73 mg/L in trauma victims and from 0.02 to 12.35 mg/L in non-trauma cases. Norephedrine (NE) was present in the blood of 22.8% (29/127) of all cases (mean of 1.81 mg/L, SD = 3.14 and in the urine of 36.2.% (mean of 15.6 mg/L, SD = 41.12). Pseudoephedrine (PE) was present in the blood of 6.3% (8/127). More than 88% (113/127) of all cases tested positive for drugs in addition to ephedrine alkaloids, the most common being cocaine or its metabolites, and morphine, each detected in the blood of 21.3% (27/127). Blood concentrations in E+ cases, where only ephedrine alkaloids were present, and E+ cases where cocaine and/or methamphetamine were present were not significantly different ($p = 0.231$). In only eight of the 127 cases (6%) was ephedrine the only drug detected in the blood. Conclusions: (1) Blood concentrations of E+ in trauma deaths completely overlap concentrations in non-trauma deaths and cannot, in isolation be used to identify the occurrence of ephedrine toxicity. This is exactly the same situation previously observed with cocaine and methamphetamine-related deaths. (2) Based on the high blood concentrations observed in this group of decedents, especially in those dying of trauma, it seems unlikely that variations in production and quality control could have much of an impact on toxicity, since the average doses contained in supplements are too low to account for the blood levels seen in this group of decedents. (3) Norephedrine was detected in the blood in only a quarter of all E+ cases, suggesting that demethylation of ephedrine does not occur to any great degree in humans, (4) blood concentrations in cases where ephedrine and illicit stimulants were both present were not significantly different, suggesting that actions of ephedrine and other stimulants are not synergistic.

Ephedrine, Norephedrine, Polydrug Abuse

K25 The Detection of Oxycodone in Meconium Samples

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After attending this presentation, attendees will learning a procedure for the extraction of oxycodone from meconium and its detection using gas chromatography-mass spectrometry (GC/MS) is described for the first time. The abuse of oxycodone (OxyContin™) has been widely discussed in the mainstream media and it is often described as a cheap form of heroin. Following the presentation, attendees will understand the abuse potential of oxycodone and be able to analyze the drug in meconium.

The described procedure can be used by researchers to determine the exposure of newborns to oxycodone, a drug with high abuse potential. The detection of exposed neonates will aid in their treatment, and allow mothers to be counseled and assisted with their drug use problems. Identification of drug exposed newborns may assist in the prevention of further drug-addicted children being born to the same mother, since services and assistance can then be provided.

Introduction: Oxycodone is a semi-synthetic opioid derived from the opium alkaloid thebaine.Oxycodone (14-hydroxy-7,8-dihydrocodeinone) marketed as OxyContin™ and Roxicodone™ is a strong opioid agonist that is available alone or in combination with mild analgesics. It is suitable for oral and nasal administration due to high bioavailability (50-65%), which makes it a good candidate for nasal abuse. In analgesic potency, oxycodone is comparable to morphine and with the exception of hallucinations, which may occur more rarely after oxycodone than after morphine, the side effects of these drugs are closely related. The abuse potential of oxycodone is equivalent to that of morphine

Oxycodone has been reported as having a high degree of abuse and potential complications in neonates from maternal drug use. Using a standard enzyme multiplied immunoassay (EMIT) screening technology, the cross-reactivity of oxycodone to the morphine antibody is only 5-6%. A positive screening value would require a high concentration of drug to be present, so an assay for the detection of oxycodone in meconium using gas chromatography-mass spectrometry was developed. Hospitals employ routine testing of neonatal and/or maternal specimens for the determination of drug and alcohol use during pregnancy. While neonatal urine is widely tested, it gives only a short history of maternal drug use. Meconium, the first fecal material passed by a newborn, extends the window of drug detection up to 20 weeks and has become widely accepted as an alternative to urinalysis. In addition to morphine and codeine, there have been reports of heroin metabolites in meconium, hydrocodone and hydromorphone, but to date there are no reports of the metabolism, deposition or detection of oxycodone or its metabolites, in either meconium or neonatal urine. Since oxycodone has been increasingly identified as a potent narcotic resulting in drug dependence, overdose and death, its use during pregnancy may result in withdrawal symptoms in the newborn.

Sample Preparation: Deuterated internal standard (50 µL) was added to an aliquot (0.5 g) of each calibrator, control or meconium specimen. The internal standard concentration of deuterated oxycodone was 200 ng/g. Methanol (3 mL) was added and the specimens were homogenized, centrifuged, and the supernatant was decanted into a small glass tube. The supernatant was evaporated to dryness at 40°C, and refrigerated overnight. The next day, 0.1M hydrochloric acid (3 mL) was added with 250µL of 10% methoxyamine hydrochloride (aqueous). The mix was incubated at room temperature for 1 hour and mixed. 0.1M phosphate buffer (pH 6.0; 3mL) was added.

Extraction Procedure: Solid-phase mixed mode extraction columns were placed into a vacuum extraction manifold. Each column was conditioned with methanol (3 mL), deionized water (3 mL) and 0.1 M phosphate buffer (pH 6.0; 3 mL). The sample was allowed to flow through the column using no vacuum. The sorbent bed was dried for one minute at full vacuum. The column was washed with deionized water (3 mL), 0.1M hydrochloric acid (3 mL) and methanol (3 mL). The column was allowed to dry after each wash stage. Glass collection tubes were placed in the manifold and the opiates were finally eluted fresh methylene chloride: isopropanol: ammonium hydroxide (80:20:5; 3mL). The extracts were evaporated to dryness under nitrogen at 17psi at 60°C. Ethanol (100µL) was added, the specimens were mixed and transferred to autosampler vial inserts and re-evaporated to dryness.

Derivatization: The vials were capped and the residue was reconstituted with 50 µL iso-octane and 10 µL of BSTFA + 1% TMCS. The extracts were heated for 30 minutes at 80°C in dry heating block prior to analysis by GC/MS.

Analytical Procedure: An Agilent 6890 gas chromatograph coupled to a 5973 mass selective detector (MSD) operating in electron impact mode was used for analysis. The gas chromatographic column was 5% phenyl -95% methyl silicone DB-5 MS, 0.20 mm ID, 0.33 µm film thickness, 25 m length and the injection temperature was 280°C. The injection mode was splitless and the injection volume was 3 µL. The oven was programmed from 150°C for 1 minute; ramped at 20°C/min to 245°C and held for 8 minutes. Then, it was ramped at 50°C/min to 290°C. The source was held at 230°C and the quadropole at 150°C. The ions monitored were 419.4, 420.4 for d_3 Oxycodone and 416.4, 417.4 for Oxycodon.

Results and Discussion: Since inception of this procedure, three meconium specimens received into our laboratory have been determined as being positive for oxycodone. The concentrations detected were 117 ng/g, 150 ng/g and 2279 ng/g. There are no published reports of oxycodone concentrations in meconium samples, so correlation with maternal use or abuse is not possible.

While no specific reports of oxycodone on neonatal outcome have been reported, there are many papers studying the effects of heroin, buprenorphine, methadone or other opiates. It is estimated that 55-94% of infants born to opioid-dependent mothers in the USA show signs of withdrawal. Newborns exhibiting neonatal abstinence syndrome (NAS) generally have longer hospital stays and symptoms include tremors, irritability, sleep abnormalities, feeding problems, low birth weight and seizures. It has further been shown that methadone maintenance treatment during pregnancy is associated with more consistent prenatal care, more normal fetal growth and reduced fetal mortality. However, neonatal withdrawal from methadone appears to be more severe than from heroin, as judged by amount of medication required to control symptoms and duration of treatment. The analytical procedure described details the determination of oxycodone in meconium specimens and may provide useful information to neonatologists and researchers studying the effects of opiates on newborns.

Oxycodone, Meconium, Neonatal Abstinence Syndrome

K26 Distribution and Comparison of Oxycodone and Other Drugs in a Case with Pre- and Post-Embalmed Autopsy Specimens

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This poster will provide the reviewer a potential means of correlating oxycodone, acetaminophen, paroxetine and alprazolam concentrations in pre- and post-embalmed autopsy specimens.

The data presented can provide potentially useful interpretive information for several drugs in pre- and post-embalmed blood and tissues.

Embalming fluid is a formaldehyde-based fluid that is infused into the body through the vasculature in preparation for burial to help disinfect and preserve the remains. It is well-recognized that embalming fluids can alter many agents of toxicological significance. For example, pseudoephedrine in the presence of formaldehyde partially or completely converts to an oxazolidine structure. At its worst, the effects of embalming fluids on cyanide may result in an inability to detect the compound at all in postmortem specimens. Unfortunately, there is a general paucity of information regarding the effects of embalming on the majority of compounds of toxicological interest. Most studies on the subject involve in vitro experiments. The work here afforded us a rare opportunity to study the effects of embalming on a few different drugs before and after embalming in the same individual.

The case history was that of a 20-year-old male who had undergone a dental procedure. For pain, he was prescribed an oxycodone/acetaminophen compound. He was subsequently found dead at home within 24-hr post-procedure.Within a period of three days, he was autopsied, embalmed and then re-autopsied. Typical tissue specimens were collected during both autopsies. In addition, while whole blood was not available during the second autopsy, a blood-like substance was recovered from the left popliteal vein and submitted for toxicological analysis.

Two different laboratories performed the toxicological analyses; with one laboratory analyzing pre-embalmed specimens while the other analyzed post-embalmed specimens.Analyses were carried out using standard extraction and analytical toxicological testing procedures and followed the individual laboratories standard operating procedures. In addition, post-embalmed specimen analyte concentrations were determined by the method of standard addition and dilution. Analytical techniques included liquid chromatography, gas chromatography and gas chromatography/mass spectrometry.

Oxycodone Concentrations in Pre- and Post Embalmed Fluid and Tissues

Specimen	Pre-Embalmed Levels	Post-Embalmed Levels
Blood	500 ng/mL	120 ng/mL*
Liver	400 ng/g	720 ng/g**
Kidney	NP	1800 ng/g**

*blood-like material from popliteal vein
**total concentration

Other pre- and post-embalmed drug findings.

Pre-Embalmed Concentrations

Specimen	Acetaminophen	Alprazolam	OH-Alprazolam	Paroxetine
Blood	< 10 mcg/mL	60.9 ng/mL	< 10 ng/mL	200 ng/mL
Liver	Not Detected	245 ng/g	24 ng/g	9.2 mcg/g

Post-Embalmed Concentrations

Specimen	Acetaminophen	Alprazolam	OH-Alprazolam	Paroxetine
Blood	0.57 mcg/mL	46 ng/mL	----	~ 140 ng/mL
Liver	4.3 mcg/g	410 ng/g	~ 25 ng/g	~7.6 mcg/g

This case allowed for a comparison of oxycodone, acetaminophen, paroxetine, alprazolam and hydroxyalprazolam concentrations in blood and liver as determined prior to embalming and following the embalming process. One potentially influencing factor in comparing the concentrations in this case was that two different laboratories performed the analyses. Even so, findings revealed that oxycodone in the blood decreased by 76% whereas in the liver levels increased by 80% following embalming. The latter finding can be explained through comparison of free versus total concentrations, especially in light that between 7-30% of oxycodone is excreted as a glucuronide conjugate. The former finding is most likely due to the post-embalming "blood" specimen and the effect of embalming fluids (i.e., degradation of oxycodone, redistribution of drug, dilution effects). Regardless, it appears that liver oxycodone findings may be a good monitor of pre-embalmed concentrations in post-embalmed tissue. Other drug findings in the case were comparable pre- and post-embalming and would not appear to have significant affect on the interpretation of the findings.

When comparing pre- and post-embalmed drug findings, numerous factors must be considered, including: completeness of initial tissue perfusion and pooling effects that may occur between and within specimen types; incomplete or non-uniform perfusion of any given tissue with embalming fluid; potential redistribution of drugs caused by the embalming fluids; and, analyte stability in embalming fluids. In addition, it is sometimes inferred that penetration of the embalming fluid is uniform throughout the body. However, this may not necessarily be true in all instances. For example, more vascularized tissues have greater infiltration of embalming fluid. In addition, structures closest to the site of administration are subject to higher pressures of infiltration.

The consequence of these variations in pre- and post-embalmed fluids and tissues is dependent upon factors surrounding the nature of the death investigation. One should be acutely aware that the process of embalming might affect the concentrations of certain drugs within body fluids and tissues. This study provides potentially useful interpretive information for the compounds detected in this case.

Acknowledgment: Forensic Toxicology Laboratory, Office of Chief Medical Examiner, New York, NY

Embalmed, Oxycodone, Distribution

K27 The Use of Hair Analysis in Postmortem Toxicology to Aid in the Determination of Cause of Death

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After attending this presentation, attendees will understand the potential use of hair analysis to aid in cause of death determinations.

The victim was found unresponsive and was transported to the emergency room via ambulance. Upon presentation to the emergency room on 4/14/XX at 0452 hours, the victim was in cardiac arrest with hypoxic ischemic encephalopathy, secondary to a possible drug overdose. The victim was transferred to the ICU and on 4/15/XX the parents signed a withdrawal of care form. On 4/18/XX at 0430 hours the victim was pronounced dead.

The MCCO was not notified of the death until 4/25/XX at 1435 hours. After review of the medical records and police reports MCCO requested an exhumation of the body and an autopsy was performed. Based on police reports, the decedent was a known heroin abuser. Autopsy results were remarkable for mild cerebral edema and histologic confirmation of hypoxic brain injury and diffuse ischemic injury within the liver.

The toxicology laboratory at MCCO received the following specimens; peripheral blood (1 mL), abdominal inferior vena cava and portal venous blood (35 mL), brain, cerebrospinal fluid (CSF), gastric, liver, muscle, urine (trace), vitreous fluid, bile and head hair. The specimens were from an embalmed body and there was no hospital admission blood available. Analysis of the bile and blood revealed the presence of lorazepam and midazolam. Analysis of the blood and the vitreous for opiates revealed the presence of free morphine. During the decedent's hospital course lorazepam, midazolam and morphine was administered.

Analysis of the hair was performed in an attempt to document the presence of 6-monoacetylmorphine (6-MAM), a marker of heroin use. Hair analysis was accomplished using a combination of two published methods, Welch et al., (1993) and United Chemical Technologies (UCT) Inc., in conjunction with in house development. The hair was prepared for analysis using the Welch method and was extracted by solid phase extraction (SPE) using UCT method. A blank hair matrix was also analyzed.

Hair analysis revealed the presence of 6-MAM, codeine, morphine and oxycodone. Allegations later arose which indicated that the manner of death in this case may be homicide. These allegations are still under investigation at this time. In light of the decedents' history in combination with an autopsy revealing no evidence of traumatic injury, the cause of death was determined to be acute heroin intoxication. Hair analysis played a key role in confirming previous heroin exposure thus enabling cause of death determination.

Opiates, Hair Analysis, Postmortem Toxicology

K28 Analysis of Nitrite in Adulterated Urine Specimens by Capillary Electrophoresis

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The purpose of this study was to develop and validate a simple, inexpensive, and robust method for the detection and quantitation of nitrite in urine for the confirmation of screening results. A method was developed that was specific, accurate, and precise with a wide dynamic range.

This research presents methods that improve the detection of nitrites in urine, which have been used to adulterate samples submitted for forensic urine drug testing. Thus, this presentation provides a method to make this testing more reliable.

Continual issues arise in urine drug testing with adulteration of samples. Nitrite (NO2-) compounds are sometimes used as adulterants to destroy traces of drugs in urine samples. Many laboratories use either specific or general oxidant colorimetric tests to screen for the presence of nitrite or other oxidants. However, for forensic acceptability, it is necessary to have a second test of the samples, preferably using a distinctly different chemical basis to confirm the initial findings.

A Beckman Coulter P/ACE MDQ Capillary Electrophoresis System was used for these experiments. The capillary was uncoated fused silica with an inner diameter of 0.75 mm and an effective length of 40 cm (total length of 50 cm). A window was burned in the polyimide coating with a lighter for direct UV detection at 214 nm. The method employed a hydrodynamic injection, and the analytes were separated using -25 kV. The column temperature was maintained at 35ºC by a liquid cooling system. Each buffer reservoir consisted of a 2-mL vial containing 1.3 mL of run buffer. Each reservoir was used for no more than three injections. The run buffer consisted of 25 mM phosphate with 3.5 mM TBAS as a modifier to slow the electro-osmotic flow. The pH was adjusted to 7.5 with NaOH.

At the start of each batch of samples, the column went through an initial regeneration/equilibration cycle that included washes with NaOH and buffer. Before each sample was injected, the column was flushed for 1min with run buffer. After every three samples, the column was regenerated with NaOH and buffer.

The lower limit of linearity for this method was determined to be 80 mg/mL NO2- in urine (4mg / mL on-column concentration.) Although the quantitative values were acceptable up to 6000 mg/mL, the relative migration time restricted the upper limit of linearity to 1500 mg/mL. The LOD for this method was determined to be 20 mg/mL NO2- in urine with a S:N of approximately 11.

The precision and accuracy of the method were determined by analyzing Axiom Test True™ Truetrol™ Adulteration Controls. Controls were analyzed as received, and were also diluted to span more of the linear range. The precision of the data was good with the relative standard deviations of the calculated concentrations consistently below 2%. The accuracy of these analyses was acceptable, as the concentrations obtained for all of the samples within the linear range were within + 20% of the actual values.

Several anions were studied to determine if they would interfere with the analysis of the NO2-. The anions were fortified in urine at concentrations of 100 or 1000 mg/mL, except chloride, which was fortified at concentrations of 1000, or 10,000 mg/mL. Interference with the NO2- quantitation was checked at the lower limit of linearity (80 mg/mL) and at the threshold cutoff concentration (500 mg/mL). These data indicated that, among these anions, there were no serious interferences noted at the threshold level of 500 mg/mL. However, CrO4-2, S2O8-2, and Cl- caused erroneously high results at the lower limit of linearity (80 mg/mL NO2-). None of the other anions that were tested interfered with the quantitative analysis of the nitrite.

There were two main issues noted with this method. The first was that it was difficult to attain reproducible migration times. The migration times became progressively longer with each injection, and no amount of buffer rinsing helped. It was decided to use a 1-minute buffer rinse between each injection to flush the column and replenish the electrolyte, then perform a 13-minute regeneration cycle after each third injection. This method resulted in the 2.2% RSD for the relative migration time of nitrite that was reported earlier in this paper. (The absolute migration times had approximately 7% RSD.) Regenerating after each injection could readily reduce the error in the migration time. However, it was decided that the increased precision in the migration time was not worth the large amount of time that would be required to regenerate after each injection.

The second main issue was the peak shape of both the nitrite and the internal standard. Due to differences in the mobilities of the analytes relative to the phosphate run buffer, both peaks fronted. The only means to correct this problem would be to change the buffer system. Various buffer systems were tested, but none performed well. Given the simplicity of the phosphate buffer system, the ease of making it, and the low cost of the chemicals, it was decided that the phosphate buffer provided adequate results.

The method had an acceptable range of linearity, with good quantitative precision and accuracy. The precision in the relative migration times was not as impressive, as it is recommended that a 4% window be allowed rather than the standard 2% window applied to most chromatographic methods. The method had few interferences, and the buffers and samples were simple and inexpensive to prepare. The method passed a rigorous validation protocol, and was successfully used to test more than 100 real urine samples.

Adulteration Testing, Nitrite in Urine, Capillary Ion Electrophoresis

K29 Evaluation of Data From Non-Physiological Workplace Drug Testing Urine Samples

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After attending this presentation, attendees will understand complexity related to the evaluation of pilot urine samples for specimen validity.

This presentation will provide authorities with information for carefully assessing the possibility of non-physiological sample submission and related alteration confirmation when evaluating all workplace urine drug test results.

Safety sensitive workers in the transportation industry are required by federal law to provide valid urine samples for workplace drug testing. A number of readily available adulterants may effectively disrupt such urine testing, allowing workers to circumvent this mandate. In addition,

water loading may dilute a drug below its analytical detection limit in urine. Several lawsuits involving airline personnel in such cases have already been litigated. This study documents types of altered urine samples received from aviation pilots and mechanics. During 1999-2001, laboratory litigation packages from 50 cases of suspected alterations were submitted through the FAA's Drug Abatement program to the Civil Aerospace Medical Institute for expert review. Methods from laboratories performing these drug and alteration analyses were examined for forensic defensibility. Data were evaluated for the types of urine-modifiers present in these cases. Six different types of alterations were found. There were 17 cases of adulteration with chromate, 15 with nitrite, 5 with acid, 2 with glutaraldehyde, and 1 with soap—7 of these 40 cases involved multiple adulterant additions and/or dilutions. The remaining 10 cases, out of 50 total, were only diluted or substituted, wherein creatinine concentrations were less than 20 or 5 mg/dl, respectively. In approximately 30 of the 50 cases, the initial drug assays were negative, suggesting possible masking of drug use. However, detection of non-physiological conditions flagged these particular urine samples for further testing. Drug confirmations were successful in 2 cases, even though adulterated. Alterations of urine were confirmed in all 50 cases. Donors may alter their urine in many ways. Laboratories use a wide variety of screening and confirmation assays in verifying these alterations. Therefore, aeromedical authorities must carefully assess the possibility of non-physiological sample submission and related alteration confirmation when evaluating all workplace urine drug test results.

Forensic Urine Drug Testing, Specimen Validity Testing, Specimen Alterations

K30 Compliance of Individuals Prescribed Dexedrine® Through Determination of Amphetamine Isomer Ratios in Oral Fluid

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After attending this presentation, attendees will understand the usefulness of determining amphetamine isomers in oral fluid as a means of assessing a patient's compliance with prescribed Dexedrine®.

An application of oral fluid as means of assessing an individual's compliance with prescribed Dexedrine®.

Oral fluid samples (N=20) were collected from individuals in drug treatment programmes who were prescribed Dexedrine® (N=10) or had a history of amphetamine use (N=10). Samples were collected on-site using the Cozart® RapiScan oral fluid collection system and sent to the laboratory for immunoassay screening. Amphetamine positive screens were confirmed initially by GC-MS-EI following solid-phase extraction with Bond Elut Certify columns and derivatisation with PFPA diluted 1:1 with ethyl acetate.

Oral fluid samples confirmed positive for amphetamine by GC-MS-EI were then analysed for both, the S-(+) and R-(-) isomers of amphetamine. After a simple dilution step (carbonate buffer, pH 9), oral fluid samples (0.05 mL) were derivatized with S-(-)-heptafluorobutyryl-prolyl chloride. Resulting diastereomers were extracted into 0.1 mL of cyclohexane, separated by GC (HP-5MS column) and detected by MS in the negative-ion chemical ionisation mode, with a calibration range of 75-3750 µg/L for each enantiomer of amphetamine.

Amphetamine was confirmed in all twenty oral fluid samples collected, S-(+)-amphetamine concentrations ranged from below LOQ to 3513 ng/mL and from below LOD to 1872 ng/mL for R-(-)-amphetamine. The R/S-amphetamine ratios ranged from 0.02 to 0.08 with a median of 0.05 for individuals compliant with the prescribed Dexedrine® and from 1.02 to 1.99 with a median of 1.30 for subjects using illicit amphetamine. This study has shown that determining amphetamine isomer ratios in oral fluid provides a simple and effective means of assessing an individuals compliance with prescribed Dexedrine®.

Dexedrine, Isomer Ratios, Oral Fluid

K31 An Assessment Oral Fluids Point-of-Collection Drug-Testing Devices

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After attending this presentation, attendees will understand the advantages and limitations of currently marketed oral fluids point of collection devices for use in forensic cases.

This presentation will impact the forensic community by demonstrating Toxicology testing has reached cross roads where testing of non-traditional specimens such as hair, sweat and oral fluids may replace testing of traditional specimens such as blood and urine. The data presented here will assist the audience in evaluating the potential use of oral fluids and point of collection oral fluid testing devices in forensic applications.

New technology is currently being marketed to rapidly test oral fluids for drugs of abuse at the point of collection. There are no nationally accepted standards or cutoff concentrations for detecting drugs in oral fluids [either workplace or criminal justice] and, for most analytes there are significant differences in cutoff concentrations across devices [i.e., sensitivity to detect drug]. In this study, we evaluated six devices [Oral Screen-Ansys Technologies, Inc. USA; Oratect - Brannan Medical USA, Rapiscan -Cozart Bioscience Ltd., UK; Uplink - Orasure Technologies USA/Germany, Drugwipe - Securetec, Germany and SalivaScreen -Ulti-Med, Germany] for their ability to meet manufacturers claims, and proposed federal standards for criminal justice and workplace programs.

Human oral fluids fortified with known quantities of drug/metabolite were used to test the products. Oral fluids were fortified with known quantities of drug(s) or metabolite(s) at 0, one-half, two and ten times the cutoffs proposed by SAMHSA and used to challenge the devices. GC or LC/MS verified concentrations of the fortified drugs/metabolites. Overall, the performance of the rapid point-of-collection oral fluid drug-testing devices was quite variable. Some devices performed well in the analysis of some drugs, but poorly for others. No single device consistently performed better than the others. In general, most of the devices detected methamphetamine and opiates well, but none of the devices could reliably detect marijuana [delta-9-THC] at less that 50 ng/mL. The ability to accurately and reliably detect cocaine and amphetamine was dependent on the individual device.

Results indicate that the devices evaluated in this study are not suitable for testing programs where marijuana is the primary drug of interest. Because the devices did perform well in detecting opiates and methamphetamine they may be suitable for programs where one or both of these classes of drugs are of primary interest. For programs where cocaine or amphetamines are of interest, some devices may be suitable while others are not.

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Oral Fluids, Point of Collection Testing, Toxicology

K32 Detection of Ketamine in Urine of Nonhuman Primates After a Single Dose Using Microplate Enzyme-Linked ImmunoSorbent Assay (ELISA)

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After attending this presentation, attendees will understand the analysis of urine using newly developed ELISA screening technique for ketamine and its metabolites.

The general anesthetic ketamine (Ketalar®, Ketaject, Vetalar) (KET) is used in human and veterinary medicine for induction of anesthesia for short surgical procedures and routine veterinary examination. Its illicit use by teenagers in rave parties has been reported. It has recently been identified as a substance associated with sexual assault, so-called "date-rape" drug. Sexual predators use it for the purpose of "drugging" unsuspected victims and raping them while under the influence of the drug. The objective of this study was to apply and evaluate a newly developed ELISA screening methodology for detection of KET and its metabolites in urine samples collected from five non-human primates which received a single dose of KET, and to study how long after drug administration, KET and its metabolites can be detected. The test kits and a microplate reader were kindly provided by the Neogen Corporation, Lexington, KY. The data are of great importance to law enforcement agencies and the forensic toxicology community in order to determine how long after sexual assault the urine samples can be collected from the victim to successfully prosecute the perpetrator. This study was reviewed and approved by the University of Illinois at Chicago Animal Care Committee. The aim of this study was: 1) to apply ELISA screening for detection of KET and its major metabolites directly in 20 µl of urine, 2) to increase a detectibility by extracting urine samples prior to ELISA screening, 3) to compare results from ELISA screening with previously obtained results of NCI-GC-MS analysis of urine samples for KET and its major metabolite norketamine (NKET).

Method: Urine was collected from five stumptail macaques (*Macaca arctoides*), four females (8-19 kg) and one male (17 kg) caged individually. All animals received a wash-out period of six months prior to the experiment. One urine sample was collected from each animal before KET administration. All monkeys received a single dose (5 mg/kg, I.M.) of KET. This dose represents an average I.M. dose in humans (3-8 mg/kg). Urine samples were collected from each animal for 18 hours every day (excluding weekends) up to 24 days then once every four days up to 35 days.

Urine screening procedure: The kit was first tested using urine spiked with KET or NKET at the following concentrations: 0, 5, 10, 25, 50, 75, 100, 500, 1000, 1500, and 2000 ng/ml. They were tested using the following procedure: 20µl of sample or control (positive or negative) was added to the appropriate well. 180 µl of a 1 to 180 dilution of enzyme conjugate in Neogen's EIA buffer was then added to each well. The plate was then covered with parafilm and incubated at room temperature for 45 minutes. Each well was then washed three times with 300 ?l of Neogen's wash buffer. 150 µl of Neogen's K-Blue Substrate was then added to each well and then incubated at room temperature for 30 minutes. The plate was gently shaken periodically throughout the incubation. To insure uniform color development. 50 µl of Neogen's Red Stop Solution was added to each well in order to stop the reaction. The plate was read at 650 nm. After the limitations of the kit and cross-reactivity with NKET were established, urine collected from monkeys dosed with ketamine was tested for the presence of KET.

Extraction: All urine samples (2 ml) were extracted from urine using HCX solid phase extraction (SPE) columns. To all control and study samples, 0.1 M acetate buffer (pH 4.5, 1 ml) and crude β-glucuronidase solution (50 µl) were added, and samples were incubated for 1.5 hours at 37^0C. After incubation 1.93 M acetic acid (1 ml) and deionized water (10 ml) were added. Each SPE column was conditioned with methanol (3 ml) deionized water (3 ml) and 1.93 M acetic acid (1 ml), the sample was added and the column was washed with deionized water (3 ml), 0.1 N HCl (1 ml) and methanol (3 ml). The final elution from the extraction column was achieved using methylene chloride:isopropanol:ammonia (78:20:2:, v/v/v, 3 ml). All extracts were evaporated to dryness in the stream of nitrogen, dissolved in 20 µl of the Neogen's buffer and transferred to microplates. They were treated as described above.

Results: KET and NKET were determined to be easily detectable at 25 ng/ ml. In one monkeys KET and its metabolites were detected in urine up to four days after drug administration, in two up to seven days, in one up to eleven days, and in one animal sixteen days after KET injection. Urine extraction followed by screening using ELISA methodology allowed for significant extension of the detection period in all animals from the study.

Date-Rape Drugs, Ketamine, ELISA

K33 2,5-Dimethoxy-4-n-propylthiophenethylamine (2C-T-7) Dose Response Relationship in the Rat

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Attendees will be exposed to a model for studying the pharmacological properties of 2C-T-7 in the rat.

This report will provide novel analytical and behavioral protocols for studying the pharmacological properties of 2C-T-7, a drug that has been recently detected in postmortem cases in the United States.

Sprague-Dawley rats, 200-250 gms, were given 2C-T-7 by intraperitoneal (ip) injection to establish both a lethal and a high pharmacological dose. The doses included 5, 25, 37.5, 50, 70, and 100 mg/kg with a group size of 5. The measurements consisted of behavior, body temperature, and 2C-T-7 tissue concentrations in blood, brain, lungs, liver and heart. 2C-T-7 was quantified using a liquid-liquid extraction with trimethoxyamphetamine as internal standard. Tissues were first treated with dilute perchloric acid (8% v/v) then centrifuged. The supernatant was transferred and the pH of the specimens and standards was adjusted to > 10 by addition of ammonium hydroxide. 1-Chlorobutane was added to the standards and specimens and they were placed on a rotary extractor for ten minutes and then centrifuged. The upper solvent layer was transferred to 5.0 mL conical test tubes and the solvent evaporated to dryness using a nitrogen evaporator with a water bath set at 40°C. The dried extracts were reconstituted in 50 uL of chloroform and placed in glass autosampler vials. The samples were then analyzed by gas chromatography-EI mass spectrometry utilizing selected ion monitoring. The instruments and conditions were as follows: Agilent 6890 gas chromatograph, with a 15 meter HP-1MS (100 % methyl polysiloxane), 1.0 mL per minute helium carrier gas flow, 250°C injector port, 300°C detector interface, oven 120°C ramped to 300°C at 20°C per minute. The ions monitored were 226, 255, 183 m/z for 2 C-T-7 with the 226 m/z used as the quantitation ion and 182 m/z for the internal standard.

TABLE 1. Tremors and Convulsions After 2C-T-7 Administration

Dose (mg/kg,ip)	5	25	37.5	50	100
Rats (#)	5	5	5	19	5
Tremor	0	0	2 (40%)	2 (11%)	4 (80%)
Automatisms (Jaw)	0	0	2 (40%)	5 (26%)	2 (40%)
Myoclonic Jerks	0	0	1 (20%)	4 (21%)	3 (60%)
Intermittent Myoclonus	0	0	0	3 (16%)	3 (60%)
Jacksonian-like	0	0	0	1 (5%)	1 (20%)
Tonic-Clonic	0	0	2 (20%)	5 (26%)	5 (100%)

Physiological events resulting from the 2C-T-7 are listed in Table 1. At the 100 mg/kg dose, the animals died in 19.6 ± 10.7 minutes (range 9-35) from suffocation and/or convulsions. A straub tail followed by a tonic-clonic convulsion occurred at 9.6 ± 1.5 minutes (range 7-13). The 50-mg/kg ip dose was selected as the high pharmacological dose with one hour being past the absorption phase. Only three out of 90 rats died within that time interval (30, 42,and 55 minutes). Behavioral effects occurred within 2 minutes post 50mg/kg ip dose with peak effects between 30 and 60 minutes; at 17 hours post dosing 30% of the rats still had intermittent tremor and body jerks. No obvious behavior effects occurred post 24 hours. After the 50-mg/kg ip dose there was no significant body temperature change in the 2.5 hour measurement period. A temperature elevation did not occur at any dose. A small (2°C maximum) but significant decrease occurred after the 5, 25 and 37.5 mg/kg ip dose. 25mg/kg ip dose had greater temperature effects. Lethal 2C-T-7 tissue concentrations after the 100mg/kg dose were 23.9 ± 9.3 µg/ml for blood, 21.3 ± 9.0 µg/gm for brain, for 285.9 ± 156.0 mg/gm lungs 51.4 ± 9.2 µg/gm for heart and 126.3 ± 94.6 µg/gm for liver. Lethality in the rat is an LD_{50} of 69 ± 8 mg/kg with a minimum observed lethal dose at 37.5 mg/kg.

In conclusion, a 2C-T-7 rat model has been established to study the dose-response relationship for 2C-T-7. Future research is needed to elucidate the complete pharmacological profile of this drug.

2C-T-7, Phenethylamine, Behavior

K34 Use of MDA (the "Love Drug") and Methamphetamine in Toronto by Unsuspecting Users of Ecstasy (MDMA)

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Attendees will learn that a variety of amphetamine derivatives are now being marketed to ecstasy users in Canada who request only ecstasy (MDMA) from the drug supplier. More importantly, discussion at the presentation will offer explanations for the reasons why illicit drug manufacturers are including MDA and methamphetamine in ecstasy tablets despite the absence of any specific demand for these drugs.

This presentation will alert the forensic community and the general public to an emerging trend of illicit drug use in Canada and stimulate discussion, which will attempt to provide the basis for this change in recreational drug marketing.

Background and specific aim. MDA (3,4-methylenedioxyamphetamine), previously known as the "love drug", is a synthetic amphetamine derivative, which has been used illicitly in part for its reported ability to induce a state of heightened empathy and introspection. Although MDA was a commonly used drug thirty years ago, the present drug of choice in the "entactogen" class of drugs is the related compound "ecstasy" (3,4-methylenedioxymethamphetamine, MDMA), which can be metabolized to MDA in the human. Most users of the drug "ecstasy" expect that they are obtaining MDMA.

As death has occurred following ingestion of MDA and MDMA, it is important to establish the extent of use of either drug by the public as well as emerging trends of drug use. Typically, marketing of an illicit drug is determined to a large extent by true demand. However, demand can also be influenced by the surreptitious inclusion by the clandestine laboratory of additional substances in the drug formulation, which enhance the desired effects of the drug "cocktail."

The purpose of our pilot investigation was to establish the identity of the drug(s), which are marketed and used in the Toronto area as ecstasy. For this purpose, we conducted forensic drug hair analysis of subjects who requested from their drug supplier tablets, which contain only ecstasy (MDMA). Our hair data, together with local drug seizure findings, indicate that despite an absence of any specific demand, the drugs MDA and methamphetamine are now being marketed to unsuspecting ecstasy users.

Methods: Levels of psychostimulants (ecstasy, MDA, MDEA, PMA, PMMA, methamphetamine, amphetamine, cocaine, and metabolites), ketamine, PCP, and opiates (heroin, morphine and metabolites, codeine) were measured by GC-MS in consecutive one half-inch segments of scalp hair taken from the back of the head of 21 drug users who reported that ecstasy (MDMA) was the only drug requested over the period of time corresponding approximately to the extent of growth of scalp hair (one month/one half-inch of hair) with the exception, for some of the subjects, of use of cannabis and "mushrooms."

Major Findings; MDMA was detected in one or more one-half inch hair segments of 19 of the 21 drug users, providing good agreement between the results of a structured interview and the forensic drug analysis.

MDA could be detected in most of the hair samples, which tested positive for MDMA. In urine, autopsied brain, and hair of ecstasy users, the ratio of MDA to MDMA is approximately 0.20 or lower. Although no precise cut-off ratio has yet been established, high ratios MDA:MDMA (e.g., >1.00) are highly suggestive of use of both MDA and MDMA. Thus, of the 19 subjects testing positive for MDMA, 12 subjects had MDMA levels in hair much greater than those of MDA; or MDMA in the absence of any MDA, suggesting selective use of MDMA; whereas 7 subjects had levels of MDA equal to or much greater than those of MDMA, indicating use of both MDMA and MDA. One female subject, in particular, tested positive for MDA in a total of 23 of 26 examined one half-inch hair segments (representing about two years of hair growth), with relatively low levels of MDMA in only two segments, indicating primary or exclusive use of MDA for this extensive period of time.

Hair analysis also revealed that amphetamine/methamphetamine was detected in a total of 8 of the 21 subjects.

Analysis of contents of seized suspected ecstasy tablets in the southern Ontario area confirmed presence of MDA and methamphetamine alone or in combination with MDMA. Estimates are that about half the tablets submitted for analysis in Ontario as ecstasy contain only MDMA. The remainder contain one, a few, or several of the following components: MDMA, MDA, MDEA, methamphetamine, ketamine, caffeine, ephedrine, pseudoephedrine, and/or phencyclidine. Eleven clandestine laboratories producing either MDMA or MDA were seized in southern Ontario during the time period January 2000 to June 2003. Of those eleven, three produced only MDMA; three only MDA; three MDMA, MDA, and methamphetamine; and two MDMA and methamphetamine.

Conclusions: Ecstasy users need to be advised that MDA and methamphetamine are being marketed as ecstasy despite the absence of any specific demand for these amphetamine derivatives. Possible reasons for the clandestine laboratory operators to include MDA and/or methamphetamine in their "ecstasy" preparations are related to either the syntheses involved or the effect that the added (substituted) drugs will have on the user.

Conversations with two MDMA / MDA clandestine lab operators indicated that they perceived that the preparation of MDA is simpler than that of MDMA. In addition, they believed that the chemicals needed to synthesize MDA were easier to obtain and were not monitored (by the authorities) as closely as those for the synthesis of MDMA. While neither of these statements is necessarily true, it is the underground chemist's perception that is important. It is also possible that MDA is included in some ecstasy preparations because of its reputed higher potency and longer half-life. Methamphetamine might be included in ecstasy tablets to provide enhanced stimulant effects and to maintain and increase market size because of the addiction potential of the drug.

Finally, our observations confirm that studies designed to determine whether ecstasy alone might cause brain damage need to confirm by forensic drug testing whether ecstasy was the only illicit drug used by the subject.

The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting views of the United States Departments of Army or Defense, or of Health Canada.

Ecstasy, MDA, Methamphetamine

K35 Postmortem Morphine Concentrations – Are They Meaningful?

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The goals of this presentation are to review factors influencing postmortem morphine concentrations and to compare concentrations in terminally ill patients to a varied population. The presentation will provide recommendations for interpretation of postmortem morphine concentrations, as well as, describe how these factors can influence the cause and manner of death.

Introduction: Morphine is a strong opioid agonist that has become the drug of choice for the treatment of moderate to severe pain associated with cancer and in palliative and terminal care. One of the most daunting tasks for forensic pathologists is interpretation of the toxicological findings. This is especially difficult in decedents with multiple medical problems who receive morphine and other opioids for comfort care in the terminal stages of disease, in which high concentrations may be suspicious for euthanasia. Many factors will alter postmortem morphine concentrations. Intrinsic factors include general health, disease processes, renal failure and hepatic function. Postmortem factors include the postmortem interval, site of specimen collection, and postmortem redistribution. Medication factors include dosing, frequency, duration of exposure to opioids and tolerance to opioids. Published therapeutic and toxic values of morphine are typically based on measures in living, non-chronic users, and postmortem data obtained from terminal patients receiving morphine for comfort measures is lacking.

Methods: We conducted a retrospective review of medical examiner cases with morphine identified in the toxicological evaluation. More than 50 cases from the District 5 Medical Examiners Office in Leesburg, Florida from the years 2001, 2002 and 2003 were identified. Included were deaths that occurred at home, with and without hospice care; in nursing homes and assisted living facilities, with and without hospice care; and deaths that occurred in a hospital, inpatient setting. Cases involving heroin use, those with incomplete medical records, or decedents who were embalmed were excluded from the study. Antemortem medical records were reviewed for age, general health status, and disease processes with special attention to evidence of renal and hepatic failure. In addition, medication schedules and dosing were reviewed, as well as, length of time receiving morphine and previous exposure to opioids. The autopsy files were reviewed for cause and manner of death, confirmation of disease processes, site of specimen collection and the time interval from death to acquisition of specimens (postmortem interval).Toxicological analyses were performed according to standard laboratory protocol using gas chromatography-mass spectrometry for identification and quantitation of morphine. Morphine concentrations in blood were measured as free and total morphine.

Results: Evaluation of the data revealed an age range from mid 40s to early 90s. Disease processes were highly varied including cancer, dementia, acute injury and chronic pain due to injury and other causes. The data showed a wide range of morphine concentrations from less than the defined therapeutic values to more than 20 times the therapeutic value (as compared to non-chronic users). The reported cause and manner of death varied from natural death resulting from end-stage disease processes to accidental deaths from injury and morphine toxicity. The results of our study mirror previous studies with elevated postmortem morphine concentrations in decedents with renal failure, chronic use of opioids and collection of specimens from central sites. Morphine concentrations were highly variable in decedents who were terminally ill and receiving morphine for comfort measures.

Conclusion: It is important for forensic pathologists to be aware of all factors that influence postmortem morphine concentrations before deciding how these values influence the determination of cause and manner of death. Postmortem morphine concentrations were elevated in decedents with renal failure because of a decreased ability to excrete the drug. Decedents with chronic illness, cancer, and liver failure had a decreased ability to metabolize the drug. Decedents with tolerance to opioids had higher postmortem concentrations beyond the defined therapeutic range. Specimen site must also be considered, as concentrations are higher when collected from a central site versus a peripheral site. Special attention to these variables is required when the decedent was terminally ill and receiving morphine for comfort measures, as the concentrations are highly variable. The significance of a prolonged postmortem interval in these cases is unknown.

In summary, when an elevated postmortem concentration of morphine is reported, an exhaustive search of the medical records must be conducted. Information obtained should include underlying disease processes, medication schedules and dosing and evidence of length of time on morphine, previous exposure to opioids and development of tolerance. Postmortem interval should be noted and communicated to the forensic toxicologist. This information, when considered, will be important when declaring the cause and manner of death.

Morphine, Postmortem, Interpretation

K36 A Review of Succinylmonocholine Concentrations in Body Fluids and Tissues Analyzed by LC-MS/MS

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The objective of this data compilation was to summarize analytical findings for succinylmonocholine from a wide variety of forensic and clinical specimen types obtained over a period spanning four years, along with a statistical analysis of the data set; also, the identification of basal levels of succinylmonocholine in normal postmortem mammalian tissues.

Demonstration of presence of succinylmonocholine in postmortem tissue samples known not to have been exposed to succinylcholine, and presentation of levels of this compound in a variety of postmortem samples.

Succinylmonocholine is the initial breakdown product of succinylcholine (succinyldicholine), a quaternary ammonium neuromuscular blocking agent often used during surgical procedures. Succinylmonocholine is rapidly generated as a metabolite from succinylcholine by cholinesterase enzymes and, more slowly, by chemical degradative processes. Very seldom is succinylcholine itself detected, due to its very short half-life in biological systems; rather, its more stable initial metabolite, succinylmonocholine, is detected. Succinylmonocholine further breaks down to succinic acid and choline, both of which are found as normal constituents in biological matrices.

Succinylmonocholine is of forensic toxicological interest because, as the initial metabolite of succinylcholine, it is potentially useful for the identification of exposure to succinylcholine, particularly in suspected poisoning cases. In conjunction with such forensic cases (as well as numerous clinical cases), this laboratory has analyzed a wide variety of specimen types for succinylmonocholine (and other quaternary ammonium neuromuscular blocking drugs including succinylcholine itself). The analyses were performed using an initial liquid-liquid extraction procedure, followed by a reverse phase ion-pairing solid phase extraction procedure. The final extracts were analyzed for neuromuscular blocking agents by high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS), using either a tandem quadrupole instrument or a hybrid tandem quadrupole-time of flight (Q TOF) instrument.

Analytical samples varied widely in age and condition, ranging from relatively fresh clinical specimens to embalmed, exhumed specimens up to ten years old. Specimen types included: kidney, liver, brain, diaphragm, lung, urinary bladder, spleen, psoas muscle, urine, blood from various sources, skin, buttock, thigh muscle, mixed tissue, right biceps, clots from various locations, fat, kidney fluid, liver fluid, serum, renal medulla, renal cortex, formalin, plasma, bile, and bone marrow. More than 250 individual specimens were analyzed over a four-year period. Analytical findings ranged from none detected (with a typical detection limit of 1 ng/g) in most specimens to an extreme value of 7400 ng/ml in a clinical urine sample. Data from all of these analyses will be presented along with statistical analysis of the data set. Of significant interest is the identification of basal levels of succinylmonocholine in fresh autopsy specimens from individuals known not to have been exposed to succinylcholine prior to death. Typical values for these specimens were in the 10-30 ng/g range for postmortem human liver and kidney, with similar values also observed for bovine and porcine tissues. We are not aware of any prior literature documenting the presence of succinylmonocholine in normal postmortem mammalian tissues.

Succinylmonocholine, Postmortem, Tissue Concentrations

K37 Postmortem Ropivacaine (Naropin) Concentrations

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After attending this presentation, attendees will understand post-mortem concentrations of ropivacaine in biological fluids and tissues of an accidental overdose during a routine surgery involving an axillary block.

To report the concentrations of ropivacaine found in post-mortem tissue and fluid samples from a patient who died after being administered ropivacaine in a surgical setting. This is to provide post-mortem concentrations in the literature for ropivacaine, a drug that thus far has not been reported in this context.

Introduction: Ropivacaine is a local anesthetic indicated for surgical and acute pain management. It is manufactured by AstraZeneca under the name Naropin® and received FDA approval in late 1996. Ropivacaine is an amide local anesthetic, belonging to the same group as mepivacaine and bupivacaine, and is supplied as the enantiomerically pure *S*-isomer in isotonic solutions (0.2-1.0 % w/v) of the hydrochloride salt. Ropivacaine has similar potency and duration of action to bupivacaine but has less central nervous system (CNS) and cardiovascular toxicity. Ropivacaine is used as an epidural injection for surgery, obstetric procedures, and postoperative pain. Additionally, it is used in the technique of peripheral nerve block and local infiltration for surgical procedures.

A case study is presented to document the post-mortem concentrations of ropivacaine found in an individual who was to undergo open reduction and external fixation surgery on his left hand. The deceased, a 32-year-old, healthy, male Caucasian, was administered midazolam and fentanyl for sedation prior to entering the Operating Room (OR). Once in the OR, he was given ropivacaine (30 mL of a 0.5% solution; 150 mg) via an axillary block whereupon he began to seize and subsequently die in the recovery room despite 2 hours of resuscitative work, which included bicarbonate, epinephrine, and electrical shocks.

Methods and Results: Ropivacaine was extracted from the samples by liquid-liquid extraction with *n*-butyl chloride: ether (4:1) after basifying the matrix with ammonium hydroxide. Extracts were back-extracted with 1M sulfuric acid, which was further washed with hexane. After re-basifying the aqueous phase, ropivacaine was extracted with *n*-butyl acetate. The extracts were analyzed by electron ionization gas chromatography/mass spectroscopy, operating in the selected ion monitoring mode, utilizing lidocaine as the internal standard. The following groups of ions (*126*, 84, 98) and (*86*, 120, 91) were monitored for ropivacaine and lidocaine, respectively, with a calibration curve ranging from 0.1-2 mg/L.

The results of the toxicological analyses performed for ropivacaine are shown in the table below. The method of standard addition (SA) was utilized for the liver and bile. The central blood was also found to contain therapeutic concentrations of midazolam (0.05 mg/L) but fentanyl was not detected. The empty vial of ropivacaine HCl used in the OR and an unopened bottle (0.5 %) of the same lot number were also submitted for toxicological analysis. The results of the pharmacy samples were positive for ropivacaine and 4400 mg/L ropivacaine, respectively, as expected.

Source of Sample	Aorta	Femoral	Liver (SA)	Vitreous Humor	Bile (SA)
Ropivacaine	2.4 mg/L	2.0 mg/L	4.4 mg/kg	1.4 mg/L	1.9 mg/L

Discussion: Ropivacaine was developed in response to the incidence of death from several accidental intravascular injections of bupivacaine. The rationale for developing ropivacaine (the propyl analog of bupivacaine) was a drug of lower lipid solubility to bupivacaine would be less cardiotoxic. Unintended intravenous injection may, however, still cause severe CNS- and cardiac toxicity. Systemic plasma concentrations of ropivacaine depend on the total dose and concentration of drug administered, the route of administration, and the vascularity of the administration site. The recommended dose of ropivacaine is between 75-300 mg for most surgical anesthesia (epidural and major nerve block).

CNS symptoms of ropivacaine toxicity occur before cardiovascular symptoms and include numbness of the tongue, lightheadedness, visual disturbances, muscular twitching, tinnitus, and more seriously, convulsions and coma. Cardiovascular toxicity is a result of depressed cardiac conduction due to inhibition of inward flow of sodium ions, which may lead to ventricular arrhythmias and cardiac arrest. The greater tolerance to ropivacaine compared to other local anesthetics, however, may hide the early warning signs of toxicity. In two human studies (n=24) of continuous intravenous infusions of ropivacaine at a rate of 10 mg/min up to a maximum dose of 160 mg, symptoms of CNS toxicity occurred at plasma concentrations between 0.5 and 3.2 mg/L. Minimal cardiovascular effects were observed during these studies and included increased heart rate and arterial pressure.

The concentrations of total ropivacaine found in the post-mortem whole blood specimens of the deceased were consistent with those reported (1.0-6.0 mg/L; medium 3.5 mg/L) from the peripheral venous plasma samples (taken at various time intervals, 7-150 minutes, after administration) in six cases where inadvertent intravascular injection of ropivacaine during surgical procedures caused convulsions and cardiovascular toxicity. In addition, ropivacaine is highly protein bound (94%) and the whole blood concentrations reported here will, therefore, be more dilute compared to plasma concentrations. The patients in all the reported cases recovered. During surgical procedures, negative aspirations for blood and CSF fluid alone are not enough to ensure that inadvertent intravascular injection has not occurred; incremental administration and constant communication with the patient is essential to prevent accidental overdose.

In the absence of a history of illness, especially epilepsy and cardiovascular disease, and the timing of the seizure in relation to the administration of ropivacaine, it is probable that the levels reported here are indicative of fatal ropivacaine levels seen in post-mortem fluids and tissues due to inadvertent intravascular injection of the drug. These levels can be used in toxicological interpretations as to possible cause of death in subsequent investigations.

Ropivacaine, Local Anestheisa, Toxicity

K38 A Multi-Drug Fatality Involving the Highest Reported Level of Venlafaxine in London, UK

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Attendance at this presentation will enable the participant to study a British toxicological case involving alcohol and several other drugs including the highest recorded level of venlafaxine in London. The presentation will also enable the participant to learn how such cases are processed by the Forensic Toxicology Service in London, UK.

The case is an interesting one as it is the first multi-drug case involving such high concentrations of venlafaxine, in addition to a significant amount of alcohol, paroxetine, paracetamol and some tricyclic antidepressant drugs too. Considering the lack of any other significant autopsy findings, the results of our toxicological analyses are consistent with the assumption of a fatal overdose of alcohol and several drugs, including the highest measured levels of venlafaxine in the London area.

The Forensic Toxicology Service offers a screening and quantification toxicology service to most of Her Majesty's Coroners and Forensic Pathologists in London as well as various Police Forces and one branch of the Armed Forces. As a result, we are required to screen for a large number of prescribed and illicit drugs in post-mortem specimens followed by quantification of those detected. All analyses must be completed and our final report must be submitted to the Courts within 15 business days of the arrival of the case at the Service. This case was presented to the Service in May 2003, and involved a 50-year-old Caucasian female who had a history of mild depression during the previous 2 years and who had previously made two unsuccessful suicide attempts involving drug overdosing during the preceding two months. The Service was requested to subject the unpreserved post-mortem blood specimen of the deceased to our standard alcohol and general drug screen in order to facilitate HM Coroner in his Inquest into this death.

The blood alcohol was determined at a level of 174mg/dL. Paracetamol (i.e., acetaminophen) was found present at a concentration of 55mg/L but no salicylates were detected in the case specimen. Our benzodiazepine screen by LC-MS-MS on this specimen did not detect any benzodiazepines or metabolites. Using our standard liquid-liquid drug extraction scheme for basic (i.e., alkaline) drugs followed by gas chromatography – mass spectrometry (GC/MS), we were able to identify in the case specimen venlafaxine and its major metabolite, in addition to paroxetine, amitriptyline and nortriptyline. When quantified by GC/MS, venlafaxine measured 139.0mg/L and paroxetine measured 7.0mg/L. HPLC with UV detection was used to quantify amitriptyline and nortriptyline, which measured at 3.2mg/L and 0.9mg/L, respectively.

Venlafaxine is readily prescribed in the United Kingdom to treat depression under the trade name Effexor® tablets (37.5, 50 or 75 mg) and Efexor® XL modified release capsules (75 or 150 mg). Paroxetine is prescribed in the United Kingdom to also treat depression as well as obsessive-compulsive disorder and panic disorder under the trade name Seroxat® in tablets (20 or 30 mg) or liquid (10mg/5mL). Recently, there has been considerable debate regarding its safety. Amitriptyline continues to be a commonly prescribed sedative antidepressant in the UK on its own (non-proprietary as 10, 25 or 50 mg tablets), or in combination with perphenazine (Triptafen®, Triptafen®-M). Nortriptyline is also used to treat depression under the trade name Allegron® as 10 or 25 mg tablets or in combination with fluphenazine (Motipress® and Motival®).

The case is an interesting one as it is the first multi-drug case involving such high concentrations of venlafaxine, in addition to a significant amount of alcohol, paroxetine, paracetamol and some tricyclic antidepressant drugs too. Considering the lack of any other significant autopsy findings, the results of our toxicological analyses are consistent with the assumption of a fatal overdose of alcohol and several drugs, including the highest measured levels of venlafaxine in the London area.

Venlafaxine, Paroxetine, Multi-Drug Fatality

K39 Methadone Concentrations and Concurrent Drug Findings in Three Populations; Methadone Treatment Patients, Impaired Drivers, and Death Investigation Cases

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In this presentations, authors will compare methadone concentrations and concurrent drugs in three populations, to assist in interpretation of data.

Forensic Toxicologists are often asked to interpret levels of drugs in drivers and death investigations. Questions arise as to the significance, when the subject is in a methadone treatment program. In evaluating this data, it is helpful to know levels detected in treatment patients and the typical co-ingested drugs. Tolerance and drug-drug interactions can greatly influence the role of methadone in death or impairment.

Methadone is an important analgesic drug and also has a significant role in replacement therapy for individuals stopping opiate abuse. The significant narcotic analgesic effects of the drug, its frequent combined use with other CNS depressants, and the significant tolerance that can develop in long term users make methadone concentrations difficult to interpret. We evaluated methadone concentrations in three populations; individuals enrolled in a methadone treatment program who received daily controlled doses of methadone, individuals arrested for impaired driving who subsequently tested positive for methadone, and in deceased individuals who tested positive for methadone in postmortem toxicology screening. The treatment population was an urban population of 76

patients (50% male). Methadone doses ranged from 9mg to 250mg/day (mean 94mg, median 100mg) and were invariably administered as syrup. Samples were collected during a periodic medical evaluation, and were collected within 0-3.5 hours of administration of the daily dose. Methadone concentrations in this population ranged from 0.013 to 0.85mg/L (mean 0.29mg/L, median 0.29mg/L). These samples were collected anonymously and there was no information available regarding their medical examination. All patients however were ambulatory and many had driven to their appointments at the clinic. The second group studied were individuals whose driving or behavior had resulted in them being arrested, suspected of drug impaired driving. Over an 18 month period, 67 cases tested positive for methadone. The population was 73% male with a median age of 43. The average concentration of methadone in this group was 0.24mg/L (median 0.19mg/L), with a range of 0.015 to 0.83mg/L. Driver behaviors in this group included CNS depression, confusion, disorientation, incoordination, and physiological parameters consistent with intoxication from narcotic analgesics. We were not able to assess any relationship between drug concentrations and degree of effect. Finally, over the same 18month period, methadone was detected in autopsy samples from 224 deceased individuals (70% male, median age 43). Deaths were attributed to a variety of causes, not all drug related. The average methadone concentration was 0.48mg/L (median 0.26mg/L) within a range 0.05 to 7.4mg/L. We will present several case studies in the impaired driving and death investigation groups. It was concluded that the range of concentrations in ambulatory populations exceeded that previously reported for the normal therapeutic range (0.35 – 0.56mg/L), and that deaths were often attributed to methadone intoxication in the absence of other obvious causes at concentrations within the range frequently encountered in these other ambulatory populations. Consideration of the presence and concentrations of other co administered drugs, the individual's enrollment in a supervised methadone program, and the length of time they had been taking the drug are important factors in being able to assess the significance of methadone in death investigations.

Methadone, Impaired Drivers, Death Investigation

K40 Postmortem Fentanyl Concentrations Following High-Dose Transdermal Administration

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The authors intend to present postmortem fentanyl concentrations from a patient that was receiving high-dose transdermal fentanyl.

This presentation will report postmortem fentanyl concentrations following high dose transdermal administration. The reported case will provide information that will assist with the interpretation of postmortem fentanyl concentrations.

Fentanyl is a synthetic opioid analgesic that is approximately 50-100 times as potent as morphine. It is used as an adjunct to surgical anesthesia and for the management of chronic pain. Transdermal fentanyl patches (Duragesic®) are available for chronic pain management in delivery doses of 25, 50, 75 and 100 mcg/hr. Steady-state serum fentanyl concentrations reported for the 25, 50, 75 and 100 mcg/hr patches are 0.3-1.2 ng/mL, 0.6-1.8 ng/mL, 1.1-2.6 ng/mL and 1.9-3.8 ng/mL, respectively.

A case was received at the State of Delaware Office of the Chief Medical Examiner involving an AIDS patient who was receiving fentanyl for chronic pain. The decedent was a 43-year-old black male who resided alone in an apartment. He was diagnosed as HIV + in 1985 which had since progressed to full blown AIDS. According to his physician, he was dying from End Stage AIDS and had developed a staphylococcal infection of the spine. He suffered from severe pain and was initially receiving morphine for pain management. Approximately 6 to 7 months prior to his death, the decedent was prescribed Duragesic® patches for pain management. The dose was gradually increased and at the time of his death he was wearing eight 100 mcg/hr Duragesic® patches as well as receiving morphine and oxycodone for breakthrough pain. According to his physician, the decedent continued to work and function normally even at this high dose of medication. He often traveled out of town to visit friends and relatives. His physician also described him as a compliant patient.

The decedent was found dead in bed when his niece requested the police check on his welfare because he failed to show up for a doctor's appointment two days earlier. Early decompositional changes were present at the time the decedent was found. The decedent was brought to the OCME for examination because the investigator noted eight 100 mcg/hr Duragesic® patches on his thighs and was concerned he may have overdosed. During the postmortem examination, the specimens collected for toxicological analysis included subclavian blood, antecubital blood, liver and urine.

Fentanyl was analyzed in biological specimens by solid phase extraction followed by electron ionization gas chromatography-mass spectrometry (SIM mode). Quantitation was performed with deuterated fentanyl as an internal standard and a 6-point calibration curve ranging from 1.0-50 ng/mL. The concentrations of fentanyl in the various specimens analyzed are summarized in the table below:

Specimen	Fentanyl (ng/mL or ng/g)	Other findings
Subclavian Blood	35	Oxycodone: 119 ng/mL
Antecubital Blood	33	Oxycodone: 76 ng/mL
Liver	352	None
Urine	175	Oxycodone: 222 ng/mL

The blood and liver fentanyl concentrations in this case are significantly higher than the reported therapeutic concentrations for fentanyl. However, these fentanyl concentrations are consistent with the high dose of fentanyl that the decedent was receiving. Since the decedent was known to tolerate this high dose of fentanyl and function normally, the death was not ruled a fentanyl overdose. The cause of death was attributed to end stage acquired immunodeficiency syndrome and the manner was ruled natural. This case illustrates the importance of obtaining a comprehensive case history along with the autopsy and toxicological findings when determining cause of death.

Fentanyl, Transdermal, Postmortem

K41 Determination of Clozapine and Desmethylclozapine in the Postmortem Blood of a Schizophrenic Patient

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Attendance at this presentation will enable the participant to study a toxicological case involving the determination of clozapine and its metabolite, desmethylclozapine, in the blood of a schizophrenic patient who suddenly collapsed and died after recently switching to this medication. The presentation will also enable the participant to learn how such cases are processed by the Forensic Toxicology Service in London, UK.

This presentation is important to the toxicological and analytical community as it is the first such case in our London Service involving a possible toxicological involvement of clozapine. Our determination of

clozapine and metabolite in the case blood specimen levels appears to be in agreement with previously published data on the significant post-mortem redistribution of clozapine. Our findings, when considered in the light of data from the drug manufacturer's therapeutic drug monitoring scheme, do not support the hypothesis that clozapine was directly involved in this death, despite the apparently high concentrations of the drug and its metabolite, probably due to the significant post-mortem redistribution.

The Forensic Toxicology Service offers a screening and quantification toxicology service to most of Her Majesty's Coroners and Forensic Pathologists in London as well as various Police Forces and one branch of the Armed Forces. As a result, we are required to screen for a large number of prescribed and illicit drugs in post-mortem specimens followed by quantification of those detected. All analyses must be completed and our final report must be submitted to the Courts within 15 business days of the arrival of the case at the Service. This case was presented to the Service in November 2002 and involved a 40-year-old Caucasian female with a history of schizophrenia who was committed under the UK's Mental Health Act. The deceased collapsed and died only 15 days after switching to this medication under medical supervision.

We were requested to analyze an unpreserved post-mortem blood specimen from the deceased using our standard alcohol and illicit drug screens, in order to facilitate HM Coroner in his Inquest into this woman's unexplained death. No other biological specimens were available.

Using appropriate calibrators, the case blood specimen was screened for alcohol and determined to be negative. Similarly, paracetamol (i.e., acetaminophen) and salicylates were not detected. Using our standard liquid-liquid drug extraction scheme for basic (i.e., alkaline) drugs followed by gas chromatography – mass spectrometry (GC-MS), we were able to identify clozapine and its major metabolite, desmethylclozapine, in the blood sample. When quantified by GC-MS using appropriate calibrators and controls, blood clozapine was determined to be 0.8 mg/L and blood desmethylclozapine measured 0.3 mg/L. Using our benzodiazepine screen by HPLC-MS-MS no benzodiazepines or metabolites were detected in the blood specimen. No amphetamines, methadone, opiates or cocaine were detected in the sample.

Clozapine is prescribed in the United Kingdom as an antipsychotic drug to treat schizophrenia in patients unresponsive to, or intolerant of, conventional antipsychotic drugs. It is prescribed in tablet form (25 or 100 mg) under the trade name Clozaril®. Patients on clozapine must be closely supervised and must participate in a therapeutic drug monitoring scheme sponsored by the drug manufacturer. Amongst clozapine's side effects are cardiac disorders such as arrhythmia, pericarditis, myocarditis, delirium and tachycardia.

After reviewing the scientific literature, it became apparent that it is most unusual for a patient to collapse and die within 15 days of switching to clozapine therapy. Although the measured concentrations of clozapine and its metabolite initially appeared relatively high, it is now well established that these substances undergo significant post-mortem redistribution and their concentrations increase several-fold after death (Flanagan et. al., 2003). Our case is important to the toxicological and analytical community as it is the first such case in our London Service involving a possible toxicological involvement of clozapine. Our determination of clozapine and metabolite in the case blood specimen levels appears to be in agreement with previously published data on the significant post-mortem redistribution of clozapine. Our findings, when considered in the light of data from the drug manufacturer's therapeutic drug monitoring scheme, do not support the hypothesis that clozapine was directly involved in this death, despite the apparently high concentrations of the drug and its metabolite, probably due to the significant post-mortem redistribution.

Clozapine, Desmethylclozapine, GC/MS

K42 DUI on Sunday at the Occasion of a Disco Weekend in the City of Bern, Switzerland: DUI Case Reports

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Authors will present recent DUI cases and to learn about the strategy to face this problem. Young adults using recreational drugs during the weekend often drive cars without showing distinct signs of impairment. These drivers, however, are a road safety problem.

A discotheque located near the main rail station in Bern reopens after a break of one hour its doors on Sunday at 5:00 am. The customers are usually socially integrated smart looking people. It is well known that this discotheque is a place where drugs are sold and consumed as well. Between April 24, 2002, and April 13, 2003, the police made six specific traffic controls checking customers driving off this place.

Methods: The drivers were checked by the police at the nearby station. The police used urine tests indicating Amphetamines, Cannabis, Opiates and Cocaine and a breathalyzer. The police filled in the Police report of suspected inability to drive safely. For the positive cases the next step was the medical examination. The MD took two blood samples and filled in the report of medical examination. In the laboratory the urine screening was performed by EMIT or in special cases by GC/MS. Alcohol was determined in the blood sample by head-space GC-FID. The quantitative determination of drugs in the blood samples was performed by GC/MS (opiates, THC, THC - COOH, cocaine, EME, BE), GC-NPD or HPLC-DAD (basic drugs), GC-ECD (benzodiazepines). The results were reported to the legal authorities.

At the occasion of the six specific traffic controls a total of 74 DUID cases were detected. In 15 of these cases Alcohol was present as well. Most of the persons showed Polytoxikomanie. Cannabis, Amphetamines and Cocaine were the most encountered drugs in these cases.

DUI, Amphetamines, Cocaine

K43 Estimate of the Incidence of Drug-Facilitated Sexual Assault in the United States

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After attending this presentation, attendees will 1) develop sensitive and quick method for the detection of 60 drugs and compounds that could be used as a "date-rape" drug, 2) determine which drugs victims of sexual assault have in their system following the crime, and 3) determine the prevalence of "date-rape" drugs in sexual assault victims.

This work will help to further elucidate the prevalence of drug-facilitated sexual assault among presenting sexual assault victims. Currently, the impact of "date-rape" drugs on society is unknown and only anecdotal evidence exists. The authors hope to confirm or deny this evidence by examining a non-biased sample of sexual assault victims.

Sexual abuse of both women and men, while under the influence of so-called "date-rape" drugs, has been the focus of many investigations in the U.S. Throughout the 1990s, an alarming increase in anecdotal reports of this crime as well as in the number of scientific publications on drug-facilitated sexual assault has been observed. In a typical scenario, a sexual predator surreptitiously spikes the drink of an unsuspecting person with a sedative drug for the purpose of "drugging" and subsequently sexually assaulting the victim while under the influence of this substance. Reported substances associated with drug-facilitated sexual assault include flunitrazepam, other benzodiazepines such as diazepam, temazepam, clonazepam, oxazepam, and also GHB, ketamine, scopolamine, and many other sedative-hypnotics, muscle relaxants, and antihistamines.

There are approximately 100,000 reported cases of sexual assault in the U.S. every year. It is estimated (Bureau of Justice Statistics) that there are more than 300,000 sexual assaults every year, three times the number actually reported. At present, there is no reliable data or estimates on the fraction of sexual assaults - actual or reported - that involve "date-rape" drugs. This project was designed to estimate the prevalence of drug-facilitated sexual assaults through a random sample of 135-150 sexual assault complaints from four reasonably representative U.S. jurisdictions. Sites include a location near San Diego, CA, one in Texas, one in the state of Washington, and one in Minnesota.

Prospective volunteers are asked if they would like to participate in the study, following a protocol approved by the UIC IRB. Those who enroll answer a set of questions concerning the general circumstances of the assault and which if any drugs the victim is using or has used. Victim's urine is then collected and refrigerated. The victim is then asked to return in one week to donate another urine sample and a hair sample. All of these samples are then sent to our laboratory where they are properly stored.The samples are analyzed for all drugs of abuse (cocaine, opiates, PCP, etc.) and any drug, over-the-counter or prescription that could be used to incapacitate someone. The classic "date-rape" drugs are analyzed for using highly selective and sensitive methods previously developed by this laboratory.

Thus far, thirty sexual assault victims have been recruited at the Texas location, forty one at the San Diego site, and 19 at the Minnesota site. The total number of sexual assault victims recruited into the study as of 6/30/03 is 90. Urine samples collected from 31 victims have thus far been screened by immunoassay for the "NIDA" drugs of abuse. All positive samples were confirmed by GC-MS following extraction and derivatization if appropriate. Out of the 31 patients, 13 provided only one (initial) urine sample. Twelve out of the 13 samples were positive for at least one drug (cocaine, marijuana, opiates, PCP, barbiturates, amphetamines or oxazepam). Eighteen victims provided initial and follow-up urine sample as well as a hair sample. Seven out of 18 initial samples were positive for drugs. In 5 cases both initial and follow-up sample was positive for drugs. In two cases the initial sample was positive for drugs but the follow-up sample was negative.

All 90 victims have been screened by GC/MS for the additional 30 drugs and substances commonly associated with sexual assault. Sixty-five of the victims were positive for at least one of the thirty drugs with twenty-two being positive for three or more drugs. All positive samples will be confirmed by GC/MS and additionally analyzed for GHB and valproic acid.

Samples are still being received and all new samples will follow the same analysis procedure. Once all samples have been collected (January 2004), the epidemiological analysis will determine how prevalent the drugs are in sexual assault complainants in these populations. We will also be able to learn more about the reliability of self-reporting of drug use among sexual assault victims by comparing our results to what drugs they have admitted to using. Conclusions will also be drawn about drug use and age group, race, and geographical location. This research will help determine if "date-rape" drugs are a serious problem, or if anecdotal reports have exaggerated their use.

Date-Rape Drugs, Prevalence, GC/MS

K44 "Slim 10" - Slim Chance

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This is a fatal case of fulminant hepatic failure, probably induced by N-nitrosofenfluramine, present as an adulterant in a weight-reducing, herbal product, sold as complementary medicine. It illustrates the difficulties and medico-legal issues encountered in the evaluation of the role of a specific nitrosamine in the causation of death, in the absence of published reports on its hepatotoxicity in humans.

It is hoped that knowledge of the avoidably tragic consequences of this case might alert users of sliming agents, including herbal preparations (generally regarded by the lay public as being safe), to exercise vigilance and discernment in their choice of these products. From a forensic perspective, it also indicates the need for more extensive, multidisciplinary research into the human hepatotoxic potential of the nitrosamines, especially in the case of N-nitrosofenfluramine.

Case History**:** A 42-year-old lady developed acute hepatitis, which rapidly progressed to fulminant hepatic failure and eventual multi-organ failure, after having ingested an unknown quantity of a herbal product over a period of some 4 months prior to the onset of her illness. The product contained the following ingredients: *Herba Gynostemmae, Folium Camelliae Sinensis, Succus Aloes Folii Siccatus, Semen Raphani* and *Fructus Crataegi.* It was officially listed as a form of Chinese Proprietary Medicine (CPM) and marketed as weight-reducing capsules, under the trade name "Slim 10." The probable cause of liver failure was clinically assessed to be drug-induced and she eventually underwent total hepatectomy, with porto-caval shunting, in anticipation of an allogenic (living unrelated) liver transplant. Unfortunately, her condition deteriorated and she died <48 hours post-operatively, some 3 weeks post-admission.

Post-mortem Findings**:** The subject was deeply jaundiced and severely obese (BMI: 47.1 kgm^{-2}), with evidence of diffuse hemorrhage, including the presence of nearly 1.5 l of blood in the peritoneal cavity (which was likely to be iatrogenic in nature). The liver had been removed and was later recovered as a formalin-fixed specimen, which was markedly contracted, comprising multiple micronodules interspersed with extensive areas of dense fibrotic tissue. Histologically, there was massive necrosis of the hepatic parenchyma, such that the residual hepatocytes were disposed as nodules displaying variable cellular regeneration and ballooning degeneration, attended by florid ductal proliferation and mixed inflammatory infiltrates (CD3+, CD20-). Infective, autoimmune, metabolic, vascular, neoplastic and most other natural causes of massive hepatocellular necrosis were effectively excluded.

Forensic Toxicology**:** Analysis of the post-mortem blood samples yielded (µg/ml) fluconazole (1.8), frusemide (3.1), lignocaine (0.59) and tramadol (0.11), which would have been therapeutic agents administered to the patient during her last illness. Subsequent analysis of a sample of residual "Slim 10" capsules (purchased by the patient) revealed that it was contaminated by fenfluramine, N-nitrosofenfluramine (1.3-1.6 g per capsule), nicotinamide (13.3 - 15.6 g per capsule) and thyroid extract.

Conclusion**:** None of the herbal ingredients is currently known to be hepatoxic (indeed, *Succus Aloes Folii Siccatus* is apparently liver-protective) and much the same applies to fenfluramine, nicotinamide

(except in exceptionally high doses, exceeding 3 g per day) and thyroid extract. However, as nitrosamines are known to be variably hepatotoxic, it would be reasonable to surmise that, in the absence of a more plausible cause of liver damage, N-nitrosofenfluramine was the likely cause of massive hepatocellular necrosis in this instance. The importer of the herbal product was later convicted of contravening the Medicines Act and was subsequently found by a Coroner's inquiry to be responsible for having caused death through an act of criminal negligence.

N-nitrosofenfluramine, Massive Hepatocellular Necrosis, Sliming Agent

K45 The Presence and Distribution of the Cocaine Like Stimulant Fencamfamine in a Postmortem Case

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Authors will report the detection of fencamfamine in a Medical Examiner's case, to remind the toxicology community of the abuse potential of this substance and to emphasize the internet as a modern source of illicit substances.

This presentation gives information about a banned, cocaine like drug over the internet and emphasizes the accessibility and harm of it to the public.

Fencamfamine (FEN, 2-ethylamino-3-phenylnorcamphane, Figure 1) is a conformationally-rigid, cyclic analog of amphetamine. It is approximately one tenth as active as d-amphetamine as a releaser of dopamine but possibly more potent than cocaine in some behavioral tests. In rodent models, FEN has been shown to produce central nervous system stimulation. In humans, FEN has been claimed to increase drive, mental alertness and feelings of well-being. It has been evaluated for the treatment of fatigue and depression and, at one time, was prescribed for its psycho-analeptic properties. Abuse of FEN has been reported among athletes and it has been sold in the United States as cocaine. Experts have suggested it would be very difficult for an individual to distinguish FEN, in combination with a local anesthetic, from cocaine. FEN is not currently available by prescription and is listed as a banned substance by most athletic unions.

We report a drug-related fatality in which small amounts of FEN were detected. Death in this 51-year-old female was attributed to the combined presence in blood of hydrocodone (0.62 mg/L), alprazolam (0.2 mg/L) and sertraline (0.32 mg/L).

FEN was isolated from post-mortem specimens by extraction into 1-chlorobutane at an alkaline pH. Separations were achieved on a DB-5 capillary column (12 m, 0.23 mm id). The carrier gas (He) flow rate was 1.2 mL/min. The initial oven temperature was 50°C, rising after 1 min to 100°C (50°C /min) and held for 1 min. Oven temperature was increased at 20°C/min to 285°C and was held for 7 minutes. Under these conditions, the retention times of FEN and SKF-525A were 7.2 and 10.5 minutes, respectively.

Total ion spectra were collected over the mass range 40 to 450 amu. Chromatographic peaks corresponding to FEN and the internal standard were identified by positive matches to library spectra and by comparison of retention times to authentic reference standards. Major ions in the mass spectrum of FEN appeared at m/z 215 (m*) 98, 84 and 58.

Standard curves were constructed by analysis of drug-free blood spiked with FEN at concentrations of 0.01, 0.02, 0.05, 0.10, 0.15, 0.40 and 0.50 mg/L. Quantitation was by linear regression analysis of plots of relative peak area ratios (FEN area at m/z 98 / IS area at m/z 86) as the dependent variable and concentration as the independent variable. Triplicate control specimens containing 0.05 or 0.1 mg/L of FEN were assayed in parallel to the specimens.

Amounts of FEN in tissues were: 0.03 mg/L in blood, 0.44 mg/kg in liver, 0.02 mg/L in bile and 0.5 mg/L in urine. FEN in specimens chromatographed as a single well resolved peak, without evidence of erythro/threo isomer separation. The n-dealkylated metabolite of FEN was not observed.

In spite of the notoriety associated with FEN this appears to be the first report of its presence in a forensic case, in the readily available literature. The other unusual feature of this case is that the deceased apparently obtained this restricted substance through an internet source. The implication is that the internet must be realized as a modern venue of drug-diversion and distribution.

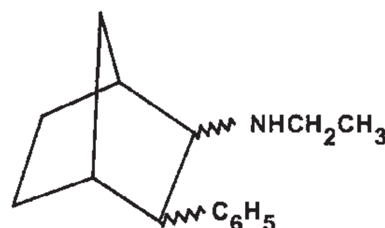

Figure 1: Chemical structure of Fencamfamine

Fencamfamine, Cocaine, Internet

K46 Acute Albuterol Intoxication in Acute Asthma: A Case Report and Review of the Literature

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After attending this presentation, attendees will take into consideration albuterol intoxication as a potential cause of death in cases of lethal asthma with resultant consideration of accident as a manner of death.

Albuterol is one of the more common therapeutic agents encountered in the routine practice of forensic pathology. While generally viewed as a medication with little potential for toxicity and even less potential for lethality, there are circumstances in which the agent itself may reasonably be considered to be the primary cause of death. This paper will present the findings in a case of acute albuterol intoxication that occurred in the setting of an acute asthma attack in a young adult male. Toxicology testing revealed a level of albuterol higher than any previously reported in the literature. This paper will also review the literature of published intoxications, both lethal and non-lethal, with reported levels. The symptoms of albuterol toxicity and its potentially lethal side-effects (hypokalemia, prolonged Q-T interval) will be highlighted.

Methods: The medical records, emergency room chart, laboratory results and autopsy protocol with toxicology results were reviewed and are presented. The medical literature was searched via PubMed using the keywords albuterol, salbutamol, toxicity, poisoning, overdose, and asthma for articles relating to acute albuterol toxicity for the period 1971 to present. Bibliographies in standard toxicology texts and articles from the initial search were also reviewed for relevant citations.

Results: Only a few cases in the literature report levels of albuterol. Of all the cases identified, the vast majority were in children and most were associated with inadvertent over dosage. Only 24 total cases reported albuterol levels and all were in adolescents and adults admitted after suicide attempt. Reported levels ranged from 50 to 449 ng/mL. The only fatality in the 24 cases also involved concomitant theophylline toxicity. None of the 24 cases involved albuterol administered in the setting of an acute asthma attack. The level in this case (490 ng/mL) exceeds any previously reported level.

Conclusions: While albuterol remains one of the safest drugs used in clinical medicine today, its overuse may not always have benign consequences. This case report highlights the potential toxicity of albuterol. Medical examiners need to be aware of the potential for drug toxicity, even in the setting of an underlying lethal disease for which the drug is prescribed. Quantitation of levels may lead to better understanding of the subset of patients that die of self-administered therapeutic overdose in the setting of acute asthma and appropriate consideration of accidental death in some cases.

Asthma, Accidental Death, Albuterol Toxicity

K47 Stability of Methadone in Frozen Postmortem Whole Blood for Five Years After Fatal Methadone Intoxication for Complex Regional Pain Syndrome

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After attending this presentation, attendees will understand that methadone is stable in frozen -20°C whole blood, and sudden death can occur after initiating methadone therapy for chronic pain syndromes.

Recognition that methadone use is increasing in clinical chronic pain syndromes, sudden death can occur from such use, and that medicolegal re-testing of frozen autopsy whole blood is valid if court ordered.

Methadone is a synthetic opioid narcotic commonly used for the treatment of heroin addiction. It was developed in the 1940s in Germany as a substitute for morphine, but its unpredictable and variable biologic behavior and half-life between patients soon rendered it unsuitable for clinical use. Today methadone is increasingly used in the treatment of chronic pain due to its longer action than other opiates, and also possibly due to the recent adverse publicity of long-acting oxycodone preparations. We present a case of fatal methadone intoxication occurring in a 39-year-old white man who suffered a work-related injury to his right ankle with development of Complex Regional Pain Syndrome (CRPS) that required toxicology re-testing for methadone five years later. Six days prior to death, the deceased had a spinal stimulator for pain control removed, and 10 mg twice daily oral methadone was added to his medication regimen. His stable chronic medications were trazodone, gabapentin, fluoxetine, baclofen, and dicloxacillin for which he was compliant. Six days after initiating methadone therapy, his wife noted he was lethargic and somnolent sitting on the couch watching television. Before she went to bed she noticed him apparently sleeping in the same position with unusual snoring respirations. The next morning she found him dead in the same position on the couch with abundant white edema foam on his face. Postmortem examination showed a well-developed and well-nourished 208 pound, 5-foot nine-inch white male with facial edema foam, and a markedly edematous, hyperpigmented and scaled right foot and ankle of CRPS. Internal examination showed cardiomegally without dilatation (534 gm), recannalized atherosclerotic-thrombotic occlusion of the left anterior descending coronary artery, anterior left ventricular wall interstitial myocardial fibrosis with few scattered lymphocytes of a healing infarct, marked pulmonary edema and congestion with airway foam. The stomach contained brown food without pill material.Toxicology testing in whole blood showed methadone in the blood and liver of 0.46 ug/ml and 1.42 ug/gm, fluoxetine 0.70 ug/ml and 10.8 ug/gm, norfluoxetine 0.69 ug/ml and 10.4 ug/gm, trazodone in blood of 0.95 ug/ml, and gabapentin in blood 6.5 ug/ml. Methadone concentration in the blood was within fatal range of 0.4-1.8 ug/ml, although levels in high-dose methadone maintenance therapy in opiate tolerant patients can overlap. Gabapentin was three times normal levels for his dose. Medication bottles were inventoried and pill counts documented with appropriate pill numbers. The cause of death was certified as methadone intoxication due to CRPS due to an industrial accident. Healing myocardial infarct due to coronary atherosclerosis was considered a significant contributing factor in his death. His pain control regimen may have prevented symptoms of his healing infarct. The manner was certified accidental. His wife filed lawsuits after workmen compensation death claims were rejected. Autopsy whole blood was saved frozen at -20° Celsius in our toxicology lab in sodium fluoride for an extended time. Numerous outside physician consultant opinions regarding cause of death were generated for all parties in the lawsuits. After five years of frozen storage the blood was re-tested through court order by an independent laboratory for methadone and metabolite EDDP. Blood methadone concentration was stable at 0.41 ug/ml and metabolite EDDP 0.064ug/ml. Lawsuits were settled after this second testing apparently confirmed that methadone can rise to known fatal levels in low dose therapy for chronic pain in a non-opiate tolerant person. Possible mechanisms for this remarkable elevation are either genetic deficiency of the enzyme that metabolizes methadone in the liver, or a competitive drug interaction, or a combination of both. These factors are important as methadone is increasingly being used as a long-acting alternative to sustained-release opiates for chronic pain treatment. A rediscovery of methadone's unpredictable biologic behavior may be repeating fifty years later. If lawsuits do arise from methadone intoxication, as have occurred in other drugs used to treat chronic pain, it is important to know that methadone is stable in frozen postmortem blood for at least five years if confirmation re-testing is ordered.

Methadone, Complex Regional Pain Syndrome, Frozen Whole Blood

K48 Acute Selenium Poisoning in a Suicide

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The authors intend to present the latest clinical and forensic science information regarding deaths attributed to acute selenium intoxication.

Selenium (Se) is a gray non-metallic chemical of the sulfur group widely used in commercial applications and therefore, a source of common human exposure. Industrial usage includes solar energy, semiconductor processing, and the manufacturing of electronics and ceramics. It is present in steel and copper alloying, metal pigmentation used in glass and paint manufacturing, rubber vulcanization, nutritional supplements, Selsun™ shampoo, and in gun-bluing agents. Selenium is also an essential mineral in the daily human diet, as there are cases of selenium deficiency syndrome.

Cases of acute selenium intoxication, as reported in the medical literature, are rare. The authors present a case of a fatal acute selenious acid intoxication covering clinical presentation prior to death, pertinent autopsy findings, and postmortem tissue concentrations. In this case, death resulted from suicidal ingestion of a gun-bluing agent by a young adult. The patient initially experienced nausea and vomiting, followed

by pulmonary edema and a rapid cardiovascular collapse approximately 3 to 4 hours post-ingestion. Postmortem toxicologic analysis of whole blood yielded toxic levels of selenium. In addition, tissue samples of brain, kidney, and liver contained high levels of selenium.

Selenium, Intoxication, Acute Poisoning

K49 Deaths Due to the Acute Effects of Drugs Investigated in Jefferson Parish Louisiana

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After attending this presentation, attendees will understand quantitative toxicology's role in rendering cause and manner of death in any case; the impact of prosector variation in under- or overestimating deaths due to the acute effects of drugs.

This presentation illuminates the growing problem of drug abuse in suburban and rural areas including the possible change in the pattern of abuse and offers the hope for/opportunity for future collaborative study with other areas, and also stresses the importance of investigative information in rendering the appropriate cause and manner of death.

The Jefferson Parish Forensic Center is a regional Forensic Center in Harvey, Louisiana that serves Jefferson Parish (JP), a suburban area neighboring New Orleans, as well as 20+ other rural and suburban parishes in south/central Louisiana. Of the 798 post mortem examinations performed in 2002, 146 (18%) were due to the acute effects of drugs. In JP alone 24% of cases (101/421) were due to the acute effect of drugs. These drug-related deaths accounted for 37% (101/272) of all violent deaths in Jefferson Parish. Of these 101 drug deaths in JP, 85 were classified as accident, 13 as suicide, and 2 as undetermined. The accidental acute drug-effect related deaths accounted for 53% (85/161) of all accidental deaths in JP. Most of these were due to the effects of multiple drugs ranging from illicit drugs such as cocaine, methamphetamine and heroin to the commonly abused prescription drugs especially hydrocodone, carisoprodol, and alprazolam. Numerous cases also were positive for methadone alone or most often in combination with other drugs. Similar trends were present in other parishes though suicides were more frequent than in JP. Additional data will be presented on the individual drugs detected, the combination of drugs including the significance of levels, the presence of concomitant natural disease, and the interaction of various factors. Rendering of the various causes and manners of deaths in cases in which drugs are present will also be discussed including possible prosector variation in under- or overestimating these types of deaths.

Drugs, Toxicology, Deaths

K50 Death Due to Acetaminophen, Doxylamine, Dextromethorphan Toxicity in an Infant

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The primary purpose of this presentation is to discuss the inherent danger of treating neonates with medication intended for adult. This is a report of an infant death due to poly over the counter drug toxicity. After a thorough review of the medical investigator's report, the autopsy findings and the toxicology results, it was concluded that the cause of death in this case was acute multiple drug toxicity, with the manner of death being homicide.

The impact of this presentation is to point out the lethal concentration of acetaminophen, dextromethorphan and doxylamine in a neonate.

The deceased is a 5-week-old male, reported by the mother to have had a runny nose, a cough and a temperature of 101.3ºF the evening of May 8. At 7:00 am the next morning, the mother gave the infant a fever reducer before she left for work, put him to bed and left him in the care of his father. The father reported the infant was irritable all day but otherwise symptomless. At some point during the day the father gave the infant "a half a dropper" of Nyquil, the exact amount undetermined. Sometime between 4:00 and 5:00 PM the father reported that he wrapped the infant in a thin sheet and put him to bed with a bottle of formula, with the bottle being propped up on a pillow. When the mother arrived home from work at 6:30pm she was told by the father that the infant was sleeping and shouldn't be disturbed. Before leaving to run an errand at 7:00 pm, the father reportedly removed the bottle from the infant's mouth but did not check further. At 7:55 pm, the mother discovered the infant cold, cyanotic and unresponsive, with milk vomitus evident. Toxicological analysis of the blood, urine, liver blood and stomach contents obtained at autopsy were subjected to a full toxicological screening which revealed the presence of ethanol, doxylamine, dextromethorphan and acetaminophen. Volatiles were quantitated by headspace GC/FID, doxylamine and dextromethorphan were extracted using a standard basic extraction and quantitated by GC/NPD and acetaminophen was quantitated by HPLC with UV detection. The results were ethanol (0.01 g/dL), doxylamine (1.2 mg/L), dextromethorphan (0.60 mg/L) and acetaminophen (294 mg/L). The stomach contained less than 125 mg/L acetaminophen and less than 2.5 mg/L each of doxylamine and dextromethorphan. The milk in the bottle was unavailable for testing.

Deaths have occurred in children from acetaminophen at 54 mg/L. The average in 139 *adults* who died of acetaminophen overdose in combination with at least one other drug was 170 mg/L. The range in 3 doxylamine fatalities is 0.7-12 mg/L, in dextromethorphan 1.1-18 mg/L.

Further investigation revealed that the father had given the infant *adult* Nyquil rather than Children's Nyquil. Adult Nyquil contains alcohol, dextromethorphan (15 mg/15 mL dose), doxylamine (6.25 mg/dose), acetaminophen (500 mg/dose) and pseudoephedrine (30 mg/dose). Children's Nyquil contains *no* alcohol, doxylamine or acetaminophen, equivalent amounts of dextromethorphan and pseudoephedrine as the adult preparation, and chlorpheniramine (2 mg/15 mL dose).

The cause of death was reported as acute multiple drug toxicity, the manner of death was homicide.

Over the Counter Drugs, Nyquil, Infant Death

K51 A Study of Drug Detection in a Postmortem Pediatric Population

Amanda J. Jenkins, PhD, Office of the Cuyahoga County Coroner, 11001 Cedar Avenue, Cleveland, OH 44106*

By attending this presentation, the attendee will: (1) acquire data regarding drug prevalence in the young, and (2) understand some of the parameters which may affect the likelihood of detecting drugs and therefore, the utility of conducting drug testing.

With declining operational budgets resulting in decrease staff and resources, Coroner and ME offices need data in order to make informed decisions about the utility of providing comprehensive services for all cases. This presentation will provide information so that attendees may be able to assess the need to conduct toxicology testing in every pediatric case.

Deaths in the pediatric population comprise a small (in absolute numbers) part of the Coroner and Medical Examiner workload. Many young individuals who die are not autopsied and for a large proportion of those that are, little or no drug testing is performed. Therefore, in most

jurisdictions, the prevalence of drug use in the young is unknown. This study was undertaken to provide some preliminary data to answer this question.

ll pediatric deaths accepted by The Office of the Cuyahoga County Coroner (CCCO) in Cleveland, Ohio in 2002 were reviewed. Demographic information including age, gender and race was collected. Cases in which the pathologist provided the laboratory with specimen were subject to toxicological testing. The heart blood or liver was subjected to comprehensive toxicological testing which included volatiles by headspace gas chromatography; acetaminophen, salicylate and ethchlorvynol screening by colorimetry; acidic/neutral and basic drug screening by liquid-liquid extraction followed by GC-FID or GC-NPD with confirmation by GC/MS; benzodiazepine screening by GC-ECD; and modified opiate immunoassay screening. If urine was submitted, immunoassay screening for amphetamines, benzodiazepines, cannabinoids, cocaine metabolites, phencyclidine, and opiates was conducted. One limitation of the study was that unless urine was submitted, or the case history suggested exposure, decedents were not routinely screened for cannabinoids.

In 2002, there were 129 deaths reviewed by CCCO. These were divided as follows:

Age	N
0 - 1 day	33
> 1 day - 2 years	43
> 2 years - 12 years	21
>12 years - 18 years	32

There were 77 males (60%) and 52 females and 55% of the individuals were black. The majority of the deaths were classified as natural (48%), with 31 (24%) accidents, 8 suicides and 13 (10%) homicides. For 9 cases, no sample was received or the sample was of insufficient volume for testing, and 5 cases were tested for volatiles only. Therefore, 115 cases were subject to comprehensive toxicological testing. No drugs were detected in 64% of these cases (N=115). Drugs administered during medical treatment, for pain and resuscitation, accounted for a further 19 cases or 16%. These drugs included lidocaine, morphine, phenytoin, midazolam, and pentobarbital. For the remaining cases, drug prevalence in descending order was as follows- cocaine/metabolites; cannabinoids; ethanol, and carbon monoxide. This preliminary data showed that the majority of cocaine/metabolite positive cases were newborns; and all cannabinoid positive cases occurred in teenagers who had violent deaths (by accident, suicide or homicide).

Pediatrics, Forensic Toxicology, Post Mortem

K1 Urinary Excretion of α-Hydroxytriazolam Following a Single Oral Dose of Halcion®

Dong-Liang Lin, PhD, Tsun-ying Huang, BS, Hsiu-Chuan Liu, BS, and Rea-Ming Yin, BS, Institute of Forensic Medicine, Taipei, Taiwan (ROC)*

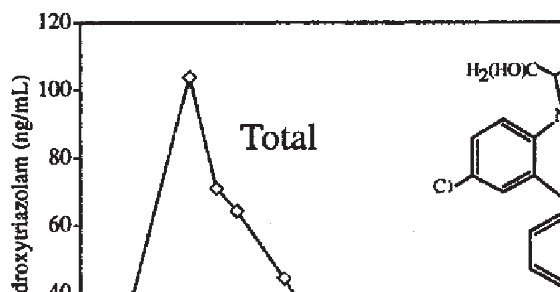
The goals of this presentation are to establish an effective procedure for analysis of a-hydroxytriazolam and to characterize human urinary excretion following of this compound following a single oral dose of triazolam.

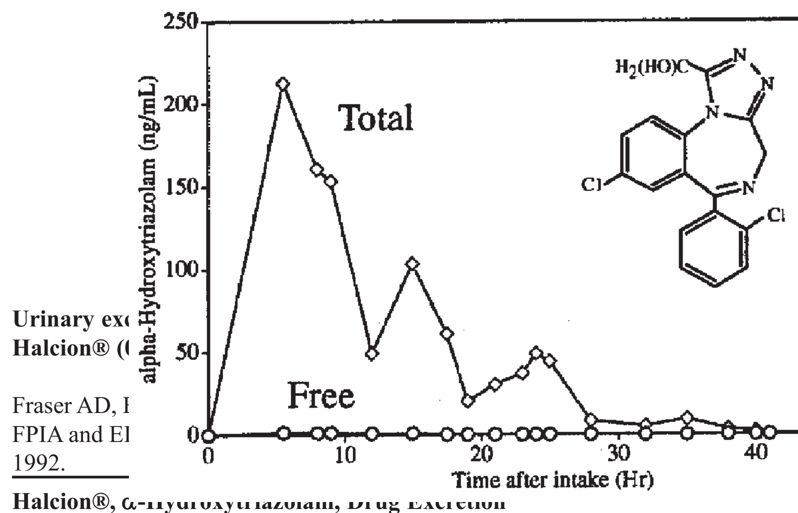
Triazolam is a very short-acting triazolobenzodiazepine with sedative hypnotic properties. Urinary excretion following an oral dose of this drug includes approximately 2% parent compound and 70% α-hydroxytriazolam glucuronide [1]. Approved for medicinal use in Taiwan, it is also controlled at the same level (Level III) as Flunitrazepam. Alleged misuses *of* this substance have been associated with case specimens submitted to this laboratory.

In this study, urine specimens were screened by TDx® followed by sample preparation (without and with enzymatic hydrolysis) and GC-MS protocols for quantitative determination *of* free and total α-hydroxytriazolam. Enzymatic hydrolysis was carried out by mixing the specimen with *Helix pomatia* β-glucuronidase for 2 hr at 56°C. The mixture was then adjusted to pH 9.5, extracted with ethyl acetate, dried, and derivatized using MSTFA. Deuterated α–hydroxytriazolam was used as the internal standard. Confirmation test was carried out using a HP 5973N GC-MSD equipped with a 30-m HP 5MS fused silica capillary column under the following conditions: Injector and interface temperature 260°C and 280°C, respectively; column oven temperature initiated at 150°C for 1 min, then programmed to 300°C at 20°C/min, and held at the final temperature for 6.50 min. Data acquisition included full-scan 50-500 amu) and selected-ion-monitoring *of* the following ions: *m/z* 415, 417, and 430 for α-hydroxyttiazolam; *m/z* 419, 421, and 434 for a-hydroxytriazolam-d_4-. Standard criteria were used to confirm the presence *of* the analyte prior to its quantitation.

The overall protocol achieved the following results when applied to the analysis of 2-mL drug-free urine specimens fortified with 10-200 ng/mL a-hydroxytriazolam: Recovery, 95%; interday and intraday precision ranges, 1.50-3.52% and 0.93-4.71%, respectively; linearity, $r^2 > 0.99$; limits of detection and quantitation, 0.05 and 0.1 ng/mL, respectively.

This protocol was applied to the analysis *of* urine samples collected from two volunteers (A and 13) taking one oral dose of Halcion® (0.25 mg triazolam). Excretion profiles *of* free and total α-hydroxytriazolam are shown in Figure 1. Free α-hydroxytriazolam is detectable, but at very low levels (<5 ng/mL). Peak excretion of total α-hydroxytriazolam occurs at approximately 5-10 hr following the drug intake. Total α-hydroxytriazolam is excreted at detectable levels approximately 2-35 hr following an oral dose *of* 0.25 mg triazolam. Total free and conjugated α-hydroxytriazolam excreted by A and B are 0.61% and 31.6%; and 0.36% and 57.2% *of* the dose, respectively.





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Halcion®, α-Hydroxytriazolam, Drug Excretion

K2 Polydrug Fatality Involving Metaxalone

Amy C. Gruszecki, DO, and Susan Kloda, MT, Department of Pathology, University of Alabama at Birmingham, 619 South 19th Street, Birmingham, AL; Gary Simmons, MD, Jefferson County Coroner Medical Examiner's Office, 1515 6th Avenue South, Birmingham, AL, and Department of Pathology, University of Alabama at Birmingham, 619 South 19th Street, Birmingham, AL; and Thomas Daly, MD, Robert Hardy, PhD, and C. Andrew Robinson, PhD, Department of Pathology, University of Alabama at Birmingham, 619 19th Street South, Birmingham, AL*

The attendee will learn how an overdose of Metaxalone, a skeletal muscle relaxant, can cause or contribute to death.

Metaxalone (Skelaxin®) [5-(3,5-dimethylphenoxymethyl)-2-oxazolidinone] is a widely used, orally administered, centrally acting skeletal muscle relaxant. It is prescribed widely for acute, chronic, traumatic, and inflammatory musculoskeletal disorders since its introduction in 1962. Metaxalone is available as 400 mg tablets with the recommended dose being 2 tablets three to four times a day.

Since its introduction, little has been reported concerning the toxicity of metaxalone in humans. A review of the literature revealed no reported cases of fatal overdose with metaxalone as a sole agent or in combination with other drugs, though it has been detected and considered non-contributory in a few case studies. In this report, the authors discuss the first reported case of a fatality in which metaxalone is felt to have played a major role.

Citalopram (Celexa®) is a selective serotonin reuptake inhibitor used to treat depression. It is administered at an initial dose of 20 mg daily, taken orally, and may be increased to 40 mg/day. Citalopram has been studied previously in fatalities and has been reported previously in a drug overdose cases. It was also detected in this case.

A 29-year-old female with a history of depression and recent ethanol abuse was found dead in a hotel room. She was discovered after she did not check out by the appropriate time. The police entered the secured room by cutting the security chain on the door. The decedent was lying on the bed. The death scene investigation found bottles of prescription drugs. Two prescription bottles for forty 400 mg tablets of metaxalone were found with directions to take one tablet four times a day. One bottle was prescribed approximately seven months prior to death and was empty. The other was prescribed approximately six

weeks prior and contained 21 tablets. Also, an empty liter wine bottle was on the nightstand. Otherwise the scene was unremarkable. No suicide note was found.

The adult decedent weighed 71 pounds and was 41 inches in length, consistent with a history of dwarfism. Postmortem examination revealed the presence of multiple subacute superficial incised wounds involving the left anterior wrist. The larynx and trachea had small amounts of edematous fluid. Particulate white granular debris was present within the duodenum and proximal half of the small bowel. The remainder of the gross and microscopic examination was unremarkable.

Urine samples were routinely screened using Syva EMIT. Samples of blood, gastric contents and liver were routinely prepared with an alkaline extraction, then analyzed and quantitated by gas chromatography-mass spectrometry. Metaxalone, citalopram, ethanol, and chlorpheniramine were identified in the postmortem samples. The concentration of metaxalone in femoral vein blood was 39 mg/L. The heart blood concentration was 54 mg/L. Femoral vein blood concentrations of citalopram and chlorpheniramine were 0.77 mg/L and 0.04 mg/L, respectively. Ethanol levels were 0.13 g/dl in vitreous and 0.08 g/dl in heart blood. Other tissue samples including brain, liver, gastric, and duodenum contents were also analyzed and were positive for metaxalone and citalopram.

The authors consider the metaxalone concentrations toxic and potentially fatal based on communications with the pharmaceutical laboratory that performs C_{max} testing for therapeutic levels of metaxalone. This laboratory reports the therapeutic level to be 4.0 mg/L in plasma under fasting conditions. The metaxalone levels in this case far exceeded this level. The citalopram concentrations found in this case were lower than those reported in fatal cases for this drug alone. Chlorpheniramine levels were also lower than those considered toxic or fatal. However, the additive central nervous system effects of toxic levels of metaxalone with citalopram, ethanol, and chlorpheniramine, also central nervous system depressants, likely caused death. Death was officially ascribed to polydrug overdose/abuse with metaxalone felt to be a major contributor. This represents the first reported case to the authors in which a metaxalone overdose significantly contributed to death.

Metaxalone, Citalopram, Overdose

K3 Interpretation of Postmortem Diphenhydramine Concentrations

Barry S. Levine, PhD, Karla A Moore, PhD, Vera Ramcharitar, MS, Russell Ramcharitar, and David Fowler, MD, Office of the Chief Medical Examiner, 111 Penn St. Baltimore, MD*

The goals of this research were to determine postmortem subclavian blood therapeutic concentrations of diphenhydramine and to compare them to reported therapeutic concentrations in living individuals. Attendees will be provided additional evidence that strict reliance on data from antemortem therapeutic concentrations to interpret postmortem blood concentrations may be risky and may lead to erroneous conclusions.

Diphenhydramine is a widely used over the counter therapeutic agent, appearing in a large number of cough and cold formulations, sleep-aids and anti-allergy medications. A recent study in Maryland identified diphenhydramine as the non-prescription therapeutic drug accounting for the most drug intoxication cases in the 1990s. Diphenhydramine continues to be frequently detected as an incidental finding in cases where death is due to other causes. Nevertheless, interpretation of diphenhydramine in these cases may have forensic relevance. A previous study in this office using heart blood specimens suggested concentrations less than 1.0 mg/L might be associated with therapeutic use.

Since the drug is alkaline extractable and has a volume of distribution of 3-4 L/kg, there is a potential for postmortem redistribution of the drug. In fact, there are a number of studies in the scientific literature that indicate postmortem redistribution of diphenhydramine does occur. To reduce the interpretive problems of postmortem redistribution, it has become common to analyze a drug from a peripheral site, such as femoral or subclavian blood instead of or in addition to heart blood. As a result, the previous study was revisited to determine whether a modification of the postmortem therapeutic range is necessary if a peripheral blood specimen is used.

Heart blood and subclavian blood specimens were quantitated for diphenhydramine in 38 cases where the medical examiner ruled that the presence of diphenhydramine was an incidental finding. Diphenhydramine was quantitated by gas chromatography-nitrogen phosphorus detection following an alkaline extraction and was confirmed by full scan electron ionization gas chromatography-mass spectrometry. A single point calibrator at a concentration of 0.8 mg/L was used for quantitation. With each batch, two blood controls at concentrations of 0.2 and 1.0 mg/L were analyzed. The limit of quantitation was 0.04 mg/L and the assay was linear to 2.4 mg/L

Thirty-four of the 38 cases had a blood concentration less than 1.0 mg/L. The average and median heart blood concentrations were 0.67 mg/L and 0.26 mg/L, respectively; the average and median peripheral blood concentrations were 0.73 mg/L and 0.25 mg/L, respectively. One case had a heart blood concentration of 9.0 mg/L and a peripheral blood concentration of 10.6 mg/L. This case was a pedestrian who died of multiple injuries. If this case is excluded, the average heart blood concentration was 0.45 mg/L and the average peripheral blood concentration was 0.46 mg/L. The average heart blood to peripheral blood diphenhydramine concentration ratio was 1.11 and the median ratio was 0.96 (range 0.27-4.34). Seventy one percent of the cases (27 of 38) had ratios between 0.7 and 1.3. The differences between the heart blood and the peripheral blood concentrations were not statistically significant. The authors conclude that the postmortem therapeutic range for diphenhydramine for a peripheral blood specimen such as subclavian blood is similar to the range established for heart blood. As a result, there are not significant interpretive differences between postmortem heart blood and subclavian blood diphenhydramine concentrations. These concentrations are slightly higher than what is reported in studies conducted in living people where concentrations up to 0.3 mg/L are reported following therapeutic use. Therefore, this study provides additional evidence that strict reliance on data from antemortem therapeutic concentrations to interpret postmortem blood concentrations may be risky and may lead to erroneous conclusions.

Diphenhydramine, Postmortem, Therapeutic

K4 False Low Bicarbonate Level With Propofol Infusion and Hypertriglyceridemia

Hisham Hashish, MD, Henry Sakowski, MD, Erica Cichowski, MD, Henry C. Nipper, PhD, and Richard Baltaro, MD, PhD, Department of Medicine, Creighton University, Omaha, NE*

The goals of this presentation are to: 1) explore the association of Propofol with fatal metabolic acidosis, 2) use the Henderson-Hasselbach equation to resolve discrepancies in acid-base analysis, and 3) recognize interfering substances that may give rise to false lab results.

A 72-year-old male who was admitted with respiratory distress and confusion was found to have a right upper lobe lung mass and hypercalcemia. His measured CO_2 level on the 1st and 2nd days of hospitalization were 25 and 23 mmol/L, respectively. On the 2nd day, he was intubated for worsening respiratory status, administered levofloxacin for

presumptive pneumonia, and administered Propofol for sedation. His arterial blood gases after intubation showed a pH 7.38, PCO_2 38, PO_2 143 and his measured CO_2 26 was mmol/L. Over the following 4 days, his measured CO_2 progressively decreased to 8 mmol/L with an anion gap of 419, negative ketones, and normal serum lactate with no corresponding significant changes in his arterial blood gases. On the 7th day of hospitalization, he received lipid infusion with total parentral nutrition. A grossly lipemic serum specimen showed a CO_2 level of 3 mmol/L. Propofol was discontinued. Four hours later, a 2nd lipemic specimen showed, after ultracentrifugation to remove the chylous material, a CO_2 level of 21 mmol/L. A lipid panel showed a triglyceride level of 4426 mgldL. The patient's condition continued to deteriorate and he died later on the 7th day. At autopsy, the cause of death was poorly differentiated small cell carcinoma in the right upper and middle lung lobes with liver and lymph node metastasis.

Propofol® is a short-acting anesthetic agent. It is a hydrophobic compound, which is formulated in a lipid emulsion (Intralipid) to facilitate intravenous use. Several cases have been reported in which an association between the use of Propofol and a clinical presentation of metabolic acidosis, cardiac dysrhythmias, and lipemia has been suggested. Some of these cases were complicated by fatality. Most of these fatal cases involved children who were ventilated for laryngotracheobronchitis. The cause of metabolic acidosis in these cases was not determined. It was also suggested that the Intralipid in the Propofol preparation might interfere with lactate metabolism in the liver causing accumulation of lactate and acidosis.

In this case, there was a consistent, progressive decrease in the measured serum bicarbonate level during Propofol infusion. However, the patient acid-base status, as simultaneously measured by arterial blood gases did not show a corresponding change that would match the very low level of serum bicarbonate. There was also a marked hypertriglyceridemia that may be related to both Propofol infusion and lipid infusion for nutritional support. After ultracentrifugation of the serum, the measured bicarbonate level in the supernatant returned to the patient's baseline value prior to Propofol and lipid administration. That bicarbonate value was consistent with the patient's acid-base status measured by arterial blood gases. Ultracentrifugation removes the chylous material from serum. It may also remove Propofol from serum since it's a hydrophobic compound. Neither Propofol nor hypertriglyceridemia have been reported as a potential interfering substance with serum bicarbonate assays. In most of the previously reported cases, metabolic acidosis, dysrhythmias, cardiac failure, and death have been related to Propofol by exclusion of all other causes. No conclusive evidence has been reported to prove or disprove this association. This is the first report suggesting that low bicarbonate level associated with Propofol infusion may be largely due to an interfering substance or substances with the bicarbonate assay. Further studies are needed to determine the role of Propofol and hypertriglyceridemia in serum bicarbonate measurement.

Propofol, Fetal Metabolic Acidosis, Bicarbonate

K5 Death Attributed to Intravenous Oxycodone

Loralie J. Langman, PhD, Henry A. Kaliciak, BSc, and Asa Louis, BSc, Provincial Toxicology Centre, Riverview Hospital, North Lawn, Port Coquitlam, British Columbia, Canada*

The authors will present the first case of death caused by intravenous oxycodone at the Provincial Toxicology Centre.

Oxycodone is a semisynthetic narcotic analgesic derived by chemical modification from codeine. It produces potent euphoria, analgesic and sedative effects, and has a dependence liability similar to morphine.

A 34-year-old Caucasian male was pronounced dead in hospital. A full autopsy was performed approximately 24 hours after death. Autopsy findings included acute bronchitis and bronchiolotis, and recent puncture sites in left arm, pulmonary edema, mucous plugging of small airways, and cerebral edema. Specimens were collected for toxicological analysis.

Blood (central) and urine specimens were initially subjected to a thorough qualitative analysis. Screening was performed for illicit drugs including morphine and cocaine by radioimmunoassay. Basic drugs were screened for by liquid-liquid extraction followed by GC-NPD and GC-MS electron impact detection. Acidic and neutral drugs were screened for by liquid-liquid extraction followed by HPLC-DAD. Volatiles were assayed by GC-FID. Qualitative analysis identified methadone, cocaine/benzoylecoginine (BE), and oxycodone. The methadone concentration was quantitated by GC-NPD and found to be 0.034 mg/L (0.11 umol/L) in blood. Quantitation of cocaine/BE was performed by GC-MS. Neither cocaine or BE were detected in blood, and no cocaine was detected in urine; however, BE was detected in urine at 0.11 mg/L (0.38 umol/L). This suggests remote cocaine useage. The methadone level was considered to be insufficient as the cause of death.

Oxycodone was assayed in biological specimens as follows: briefly, to 1 mL of specimen standards and controls 100 uL of prazepam solution (internal standard, 1.0 ug/L) and 1 mL of saturated sodium carbonate solution was added, and extracted into 5 mL n-butyl chloride. The extract was concentrated under nitrogen, reconstituted with 100 uL of methanol, and 1 µL was injected into an Agilent model 6890 gas chromatograph coupled to a NP Detector using a 30 m HP-5 capillary column (Agilent). Separation was achieved isothermally at 250°C. The concentration was measured by comparison of peak height ratio of the drug to that of prazepam against a standard curve. Since prazepam is not used therapeutically in Canada and extracts efficiently under the above conditions, it was chosen as the internal standard. Linearity was observed from 0.010 mg/L up to 0.50 mg/L. Samples with concentrations exceeding the linearity were diluted.

Elevated concentrations of oxycodone were found in blood 0.27 mg/L (0.86 mmol/L). The usual adult oral dose is 2.5-5 mg as the hydrochloride salt every 6 hours, although patients with moderately severe pain may take 10-30 mg every 4 hours. Published pharmacokinetic studies involving oxycodone show that plasma concentrations are generally less than 0.100 mg/L. For example, the peak plasma concentrations in 12 patients receiving a 10 mg oral dose averaged 0.030 mg/L. There is little reported on the lethal levels of oxycodone in blood when administered intravenously. For oral oxycodone alone, a minimum lethal level of 5.0 mg/L has been suggested, and fatal concentrations involving oxycodone and at least one other depressant drug have been reported at 0.60 mg/L. Although the concentration of oxycodone in this case was lower, it is well known that for other opiates the minimum lethal level can be considerably lower when administered intravenously than when orally administered. The cause of death in this case was ascribed to oxycodone administered by intravenous route.

Oxycodone, Intravenous, Fatality

K6 Validation and Application of the PE TMX 110 Autosystem for Packed Column Analysis of the Confirmation of Volatiles in Death Investigation

Bradford R. Hepler, PhD, Daniel S. Isenschmid, PhD, and Sawait Kanluen, MD, Wayne County Medical Examiner's Office, Detroit MI*

After attending this presentation, the attendee will be knowledgeable in method validation for volatiles by headspace gas chromatography. Documentation of linear dynamic range, precision, and carryover of volatile headspace methods on two instrumental systems

will allow the attendee to become familiar with each system. Additionally, the audience member will know how the new TMX HS 110 Autosystem compares to the older HS101 system. Data that supports the validation of the newer TMX HS110 system for use in post-mortem work will demonstrate the utility of the automated headspace approach to the audience.

Headspace Gas Chromatography has been widely applied to the determination of alcohol in postmortem specimens. In 2001, the Wayne County Medical Examiner's office (WCMEO) performed 3,572 head-space confirmation analyses on multiple samples from 3175 cases. In order to facilitate this workload automated headspace autosampling is utilized within the postmortem laboratory. The authors report here on the validation of the Perkin Elmer TMX-HS110 system for use in this application.

Validation of the TMX-HS110 system was performed by direct comparison against an existing Perkin Elmer HS101 automated system. All analyses were performed under isothermal conditions on a 6' X 1/8" OD stainless steel Carbopak B 60/80 mesh 5% Carbowax 20-M column at 80°C. The injection needle and transfer line was maintained at 110°C on each instrument. Thermostat temperatures of 60°C were utilized to heat samples before headspace injection. Injection ports and flame ion-ization detectors (FID) were maintained at 130°C. The chromatographic flow rate of the nitrogen carrier was set at 20 mL/min on each instrument, with the fuel gases hydrogen and compressed air set at 40 and 400 mL/min respectively. Purge flows on these instruments were set to values between 5-12 mL/min.

The headspace autosampler programs were consistent on each system. Thermostat time was 15.0 min, pressurization was 0.5 min, injection time 0.08 min, and withdrawal time 0.20 min. Cycle time was 7.0 minutes and each vial was vented 1 time.

All samples were prepared for analysis by diluting 0.100 mL of specimen, calibrator or control with 1.00 mL of an aqueous n-propanol internal standard solution prepared at 0.160 g/dL concentration. All patient specimens were run in duplicate. Each batch was ran by single point calibration at values of 0.1531, 0.1580, 0.1580, and 0.1562 g/dL for ethanol, methanol, acetone and isopropanol, respectively, on both analysis systems. All standards and controls were prepared in aqueous solutions. In all cases of instrumental comparison studies the same vial set was ran on each instrument in the same sequence. Linearity studies were carried out over a range of 0.007 - 1.5 g/dL for each analyte. Precision studies were performed on both systems n = 5 at up to 5 con-centrations over the linear range studied. Carryover was evaluated up to a concentration of 1.531, 1.580, 1.580 and 1.562 g/dL for ethanol, methanol, acetone and isopropanol, respectively.

Turbochrom chromatographic software was validated in parallel on the existing HS 101 analyzer against an existing LCI 100 data acqui-sition system on the HS 101-headspace analyzer. Data was collected simultaneously on the same sample set by both systems. Subsequent studies and comparisons between the two Headspace/ Chromatographic systems were performed using the Turbochrom chromatographic software.

Finally, three routine batches of patient samples were evaluated on each system. In each case the calibration sequence and sample vial sequence was identical. Analysis of the vial set was first on the HS 101 instrument (reference method), followed by analysis of the same vial set on the TMX HS110 system. In all linearity, carryover, precision and comparison studies, data reduction was performed by the Turbochrom software package using identical integration parameters.

Results of whole blood proficiency studies over an eight-year period demonstrated under the instrumental analysis conditions demon-strated consistency between mean target ranges and results obtained on the HS 101. A correlation coefficient of 0.9979 with a slope of 1.026 for n = 169 was defined. Additionally, each batch analysis includes a re-analysis whole blood sample ran as an in-house control which must meet reporting criteria (within 0.02 g/dL of original result).

Results of the data reduction system comparison between the LCI 100 and Turbochrom software packages on data collected from the HS 101 analyzer demonstrated correlation's of 0.9999 or better for ethanol, methanol, acetone and isopropanol. Standard deviations on this com-parison ranged from 0.2 - 5.4 %. From this it was concluded that data reduction from each of these systems resulted in equivalent data.

Correlation between the data on both instruments was 0.999 or better for each analyte**.** Linearity, LOD (relative retention times (RRT) within 2% of expected value and signal to noise >/= 10:1), LOQ (RRT within 2%; concentration within 20% of target value) and ULOL data for both instrument systems was determined to be equivalent and is summarized on Table 1:

Table 1: Linear ranges for headspace method, LOD, LOQ and ULOL; Ethanol, Methanol, Acetone, Isopropanol (n = 2 at each concentration).

ANALYTE	RANGE g/mL	LOD g/dL	LOQ g/dL	ULOL g/dL
Ethanol	0.0076 - 1.531	0.0076	0.0076	1.531
Methanol	0.0079 - 1.580	0.0079	0.0079	1.580
Acetone	0.0079 - 0.395	0.0079	0.0079	0.395
Isopropanol	0.0156 - 1.562	0.0078	0.0156	1.562

Carryover evaluations of ethanol, methanol, acetone and iso-propanol were determined over the linear range defined on Table 1. Carryover is noted to occur at specific concentrations documented on Table 2 at levels of 0.004 g/dL or less for all analytes.

Table 2: Carryover concentration ranges for ethanol, methanol, acetone and isopropanol.

ANALYTE	CONCENTRATION INJECTED g/dL	CARRYOVER g/dL
Ethanol	1.531	0.0018
	0.765	0.0013
	0.382 and below	None
Methanol	1.580	0.0039
	0.790	0.0018
	0.395	0.0012
	0.316 and below	None
Acetone	1.580	0.0002
	0.790 and below	None
Isopropanol	1.562	None

Precision studies performed over the linear range of the assay (n = 5 at each concentration) demonstrated accuracy within 10% of target values. All RT values were within 2% of target values. Within batch CV values over the ranges evaluated were less than 1.00, 3.60, 1.40 and 5.10 % respectively for ethanol, methanol, acetone and iso-propanol. Between run, CV values as determined by ethanol controls distributed over the range of 0.050 to 0.500 gm/100 demonstrated values of 2.60 % or less. All values were within 10% of target concentrations.

Direct batch to batch comparisons (batch size n = 73, 97, and 94) between each analytical system was run on each of three separate occa-sions by 2 analysts. Correlation's between each set of data demonstrated linear relationships with slopes of 0.96 or better and correlation coeffi-cients of 0.9995 or better. Differences between ethanol values on each of the two systems were at mean values of less than 1% with overall standard deviations of the mean value at 6% or less.

The data presented in this study demonstrate that the TMX HS110 system and the HS 101-headspace analyzer produce equivalent data. These instruments under the conditions defined can be used inter-changeably. The WCMEO postmortem laboratory currently uses both of these systems in confirmation of alcohol findings.

Headspace Analysis, Ethanol, HS 101, TMX HS110

K7 Optimizing HPLC Separation of Antidepressant Drugs Through Stationary Phase Selection

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The goal of this educational presentation is to enhance the understanding of the separation mechanisms involved in the HPLC analysis of toxicologically relevant antidepressants.

Treatment of primary depression has traditionally been accomplished using tricyclic antidepressant drugs. Over the past five years, several new antidepressant drugs have entered the marketplace and are now some of the most widely prescribed medications.

Analysis of antidepressant drugs is typically accomplished using reverse phase HPLC combined with UV detection. While the analysis of individual compounds is relatively easy, testing for antidepressants as a class or group of drugs in a clinical setting for therapeutic drug monitoring is sometimes complicated by poor or incomplete resolution of all these compounds.

Fourteen antidepressant drugs were analyzed on three different reverse phase columns. Conditions were optimized for each stationary phase for best resolution and shortest analysis time. Chromatograms illustrating the different retention mechanisms of these reverse phase columns will be shown.

Antidepressants, HPLC, Stationary Phases

K8 Amphetamine and Methamphetamine Excretion Following Administration of Multiple Doses of the Drug Gewodin®

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Following this presentation, individuals will be able to assess urine levels of amphetamine and methamphetamine for consistency with use of this medication.

Several drugs are known to be metabolized by the body to methamphetamine and/or amphetamine that are subsequently excreted in the urine. Such drugs raise concerns with interpretation of positive amphetamine drug testing results. Gewodin (Geistlich, Wolhusen, Switzerland) is a multi-ingredient medication used for pain relief. The drug contains acetominophen, caffeine, isopropylphenazone and famprofazone. The drug is available in European countries, such as Germany but is not marketed in the U.S. Famprofazone acts as an analgesic and is the component metabolized to methamphetamine and amphetamine.

Two tablets (50 mg of famprofazone) were administered orally to five volunteers with no history of amphetamine, methamphetamine or famprofazone use. Six hours following the initial dose, a second dose was administered to each subject. This was followed on the second day by administration of two additional doses, effectively administering additional doses of the drug at six, 24 and 30 hours after the initial dose. Urine samples were collected pre-dose and then ad lib after the initial dose and continued for up to seven days following the last (fourth) dose of the drug. Urine pH, specific gravity, and creatinine values were determined on all samples as was the concentration of amphetamine and methamphetamine. Drug concentrations were determined by gas chromatography/mass spectrometry (GC/MS) following liquid/liquid extraction and derivatization with heptafluorobutyric anhydride.

A previous study using a single 50 mg dose of famprofazone showed peak concentrations for amphetamine ranged from 148 - 2,271 ng/mL and 614 - 7,361 ng/mL for methamphetamine with peak concentrations of both compounds 3 - 14 hours post-dose. Using a cutoff of 500 ng/mL, all five subjects in that study had individual urine samples that tested with some of the positive samples being detected over 48 hours post-dose. The current study found peak concentrations of 5,327 - 14,154 ng/mL for methamphetamine and 833 – 3,554 ng/mL for amphetamine. Positive samples were seen for several days following the last administration of the drug.

Interpretation of results is a critical part of forensic drug testing due to the potential repercussions to an individual. As demonstrated by the current study, a positive amphetamine test does not necessarily indicate illicit drug use. Evaluation of results with regard to those found in this study will assist in determination of the possible use of this medication as the source of methamphetamine and amphetamine, particularly when multiple administrations of the drug are alleged.

Famprofazone, Amphetamine, Methamphetamine

K9 The Analysis of LSD in Urine by Quadrupole GC/MS

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The goals of this research project are to determine a fast, sensitive method for the analysis of LSD in urine

A method has been developed by which LSD in urine is analyzed by the ThermoFinnigan Trace DSQ dual stage quadrupole GC/MS. The first quadrupole is a small, bent set of rods that greatly reduces interference from neutral noise, one of the main causes of high background and hence poor sensitivity. The main analytical rods follow the bent quadrupole. The detection and analysis of LSD can be particularly difficult do to the low detection limits required for this drug. One percent or less of the parent LSD is excreted in urine and LSD dosages are in the low microgram level, therefore a method of analysis needs to reach the low pg/ml levels of detection. This is accomplished using a strong derivatizing agent, N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA), and a single ion monitoring experiment on the DSQ mass spectrometer. A calibration curve from 10 pg/mL to 500 pg/mL was run on the Trace DSQ system. A series of spiked samples in urine matrix were then extracted with solid phase extraction tubes to verify the application of the method. The collected drug was then derivatized with MSTFA and injected into the Trace DSQ system. Two microliters were injected into a split/splitless injector with a 5mm internal diameter. The volume of this liner allows for a greater injection volume without any back flash and contamination of the system. Due to the low levels required for this analysis, a full scan analysis cannot be used. Single ion monitoring allows the quadrupole to analyze one ion at a time and therefore increase the sensitivity of the analysis. The DSQ mass spectrometer was programmed for a multi ion SIM experiment. Three ions were selected, one ion for quantitation and the other two for confirmation. This paper shows that this method is able to reach the low levels required for the routine analysis of LSD in urine.

LSD, GC/MS, SIM

K10 Training in Gas Chromatography

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The participants will know the training program for gas chromatography in the Forensic Toxicology Laboratory, Office of the Medical Examiner, Cook County.

The Office of the Medical Examiner, Cook County hired recent college graduates as analysts in the forensic toxicology laboratory.

Training began with quantitation of drugs using gas chromatography (GC) with nitrogen phosphorus detector (NPD) and/or flame ionization detector (FID). Each trainee was assigned to his/her own Agilent 6890 GC. Each trainee was trained initially on either GC/NPD or GC/FID. Diphenhydramine, with dexbrompheniramine as the internal standard, was used for the GC/NPD training. Acetaminophen, with aprobarbital as the internal standard, was used for the GC/FID training.

Our program is as follows:

(1) Overview of the laboratory procedures including specimens accession, limited access storage areas, chain of evidence, case assignment, batch number system, standard/control, specimen extraction, GC analysis, result reporting and review.

(2) Operation of the Eppendorf pipets.

(3) Operation of the analytical balance.

(4) Calibration of the Eppendorf pipets.

(5) Explanation of the extraction theory, the extraction procedure and the internal standard method.

(6) Observation of the performance of a liquid/liquid extraction.

(7) Preparation of trainee's standard and control solutions.

(8) Operation of the pH meter.

(9) Preparation of trainee's buffer solution.

(10) Math test, which covered the dilutions and the arrival of extraction final concentrations.

(11) Supervised extraction of the compound of interest, with the trainee's standard and with the laboratory's standard, each in triplicate spiked in water.

(12) Explanation of the GC theory.

(13) Demonstration and practice of the GC turn on procedure, the GC performance check and the GC turn off procedure.

(14) Instruction and practice on the creation of a GC method.

(15) Instruction and practice on the preparation and running of a GC sequence, using the batch previously extracted in step 11.

(16) If good recovery and good reproducibility were achieved, the trainee would learn how to complete the paper work, otherwise, the process was repeated until satisfactory.

(17) Trainee was required to duplicate the good recovery and good reproducibility from a second batch of step 11, with minimal supervision.

(18) To extract and load on GC a batch consisting of the compound of interest, with the trainee's standard and control, as well as the laboratory's standard and control, each in duplicate spiked in water, with no supervision. Good recovery and good reproducibility were the completion criteria.

(19) To extract and load on GC a batch consisting of the compound of interest, with the laboratory's standards as calibrators, and the trainee's standards as samples. Water was the matrix. Completion criteria were acceptable calibration and the results of the samples within +/- 20% of the expected value.

(20) The trainee was required to duplicate step 19.

(21) To extract and load on GC a batch consisting of the compound of interest, with the trainee's standards and controls spiked in water. Completion criteria were acceptable calibration, acceptable positive control (+/- 20% of the expected value) and acceptable negative control (no presence of the compound of interest).

(22) The trainee was required to duplicate step 21.

(23) To extract and load on GC a batch consisting of the compound of interest, using the trainee's standards and controls spiked in water, as well as negative blood spiked with the trainee's standard in one concentration in triplicate, as samples. Completion criteria were acceptable calibration, acceptable positive control (+/- 20% of the expected value), acceptable negative control (no presence of the compound of interest), and 2 out of 3 bloods acceptable (+/- 30% of the expected value).

(24) The trainee was required to duplicate step 23.

(25) To extract and load on GC a batch consisting of the compound of interest, with the trainee's standards and controls spiked in water, as well as negative blood spiked with trainee's standards in three different concentrations, each in duplicate as samples. Completion criteria were acceptable calibration, acceptable positive control (+/- 20% of the expected value), acceptable negative control (no presence of the compound of interest), and all 3 bloods acceptable (duplicate results within +/- 10% of their average).

(26) The trainee was required to duplicate step 25.

(27) Successful completion of duplicate sets of 4 unknowns using the trainee's standards and controls spiked in water.

(28) To extract and load on GC a batch consisting of the compound of interest, with the trainee's standards and controls spiked in negative blood. Completion criteria were acceptable calibration, acceptable positive control (+/- 20% of the expected value) and acceptable negative control (no presence of compound of interest).

After successful completion of the above program, the trainee was certified to use the GC with the specific detector she/he was trained on. The newly certified analyst would be assigned to do case work. Initially, the assignment would consist of the quantitation of a single compound. Gradually, the difficulty of the quantitation would increase. All of their work was to be reviewed by themselves, a supervisor and the chief toxicologist.

Cross training between the two GC detectors involved step 13 and the successful completion of step 21, using laboratory standards and controls. The analyst was trained on GC trouble-shooting and maintenance as the situations presented themselves. They included, but not limited to, maintenance on injector port, column, detector, gas line trap, ChemStation disk, as well as handling chromatography separation problems.

After they gained more experience on the GC quantitation, they would be trained on how to do a dual column GC screening procedure. All GC analysts were trained, and their work reviewed by one person. This was intended for consistency and continuity.

Each GC analyst was paired with a GC/MS analyst. The GC/MS analyst would confirm/rule out the positive GC result quantitated by her/his partner. The GC/MS analyst would explain the GC/MS result, therefore, served as a mentor to her/his GC partner. This was intended for inspiration and possible GC/MS training in the future.

Gas Chromatography, Training, Toxicology

K11 Parameters Optimization Associated With the Analysis of Methylenedioxymethamphetamine (MDMA) and Related Compounds in Biological Matrices

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The learning objectives of this presentation are to characterize and evaluate parameters that are pertinent to the analysis of methlene-dioxymethamphetamine (MDMA) and related compounds in biological specimens.

With increasing report on MDMA abuse and required analysis, a systematic evaluation on parameters associated with the analysis of MDMA and related compounds is undertaken, including methylene-dioxyamphetamine (MDA), amphetamine (AM), and methamphetamine (MA). Parameters studied included (a) three solid-phase adsorbents; (b) five derivatization reagents; and (c) four deuterated internal standards.

Ions resulting from the use of various derivatization reagents that are potentially useful for selected-ion-monitoring (SIM) for qualitative

and quantitative determination of MDMA, MDA, MA, and AM are listed in Tables 1 and 2. TMS- and TCA-derivatives do not generate adequate number of qualified ion-pairs as required in common SIM practice. Among those generating adequate number of qualified ion-pairs, HFB-derivatives appear to produce higher ion intensities (ionization efficiencies). Some of the ion-pairs selected from the HFB-derivatives have low relative intensities in their respective spectra; however, this unfavorable factor appears to be adequately compensated for by the enhanced ionization efficiency and desirable limits of quantitation and detection still can be achieved.

HFB-derivatives of the analytes and internal standards were used to evaluate the effectiveness of internal standards. Ions adapted to designate MDMA/MDMA-d_5, MDA/MDA-d_5, MA/MA-d_8, and AM/AM-d_8 are: *m/z* 254/258, 162/167, 254/261, and 240/243. Integrated SIM intensities of these ions are used for further statistical analysis. In this study, four sets of standard solutions containing all four compounds at five low concentrations (2, 5, 10, 20, and 40 ng/mL) were prepared with all four internal standards (10 ng/mL). Another four sets with analytes at higher concentrations (100, 250, 500, 1000, and 2000 ng/mL) were also prepared (internal standard concentrations = 500 ng/mL). The first set of the four was first used as the calibrators for the calculation of analyte concentrations in the other three sets. The same process was followed by using the second, the third, and the fourth sets as the calibrators. This same process was applied to both the low and the higher concentration sets. In these calculations, MDMA-d_5 and MDA-d_5 were sequentially used as the internal standards to calculate the concentrations of MDMA and MDA. Similarly, MA-d_8 and AM-d_8 were sequentially used as the internal standards to calculate the concentrations of MA and AM. Statistical methods were then used to determine whether analyte concentrations resulting from the use of different internal standard were statistically different. Relevant statistical data are shown in Table, while the interpretation of these data are summarized in Table 4. Further studies are currently in progress to further understand the performance characteristics exhibited by the AM/MA and the MDMA/MDA pairs. These characteristics will be evaluated along with what has been observed [1] in an earlier study on the barbiturate system.

1. Liu RH, McKeehan AM, Edwards C, Foster GF, Bensley WD, Langner JG, Walia AS: Improved gas chromatography/mass spectrometry analysis of barbiturates in urine using centrifuge-based solid-phase extraction, methylation, with d_5-pentobarbital as internal standard; *J Forensic Sci* 39:1501–1514; 1994.

MDMA, MDA, Internal Standard

K12 Putting an Ecstasy Test Kit to the Test

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The learning objective of this presentation is to evaluate the reliability of the DanceSafe™ Ecstasy Testing Kit.

There has been a significant rise in the use of the club drug MDMA (3,4-methylenedioxymethamphetamine), or Ecstasy, over the past few years. Coupled with this increase in use is a rise in emergency room visits and deaths attributed to the drug. Unfortunately, many Ecstasy users feel that MDMA is a safe drug and that the problems associated with its use primarily result from contamination with other chemicals or wholesale substitution of MDMA with more dangerous drugs. As a result of this widespread belief and the media attention in the U.S., there is an ongoing effort among harm reduction organizations to provide colorimetric test kits to differentiate between substituted and authentic Ecstasy. Until recently, these kits consisted only of the Marquis reagent, a reagent routinely used by law enforcement agencies and crime laboratories. In an attempt to resolve the ambiguity in interpreting results when using the Marquis reagent as a single test, some organizations such as DanceSafe™ (www.dancesafe.org), have recently updated their test kit by adding two additional colorimetric tests, the Mecke and Simon's reagents.

As with most colorimetric methods, the interpretation of these qualitative tests is highly subjective. The danger here is two-fold. First, the users of the test are typically young and generally inexperienced with the testing procedures. Second, the tests themselves cannot reliably differentiate MDMA from other chemically-related phenethylamines, as well as other drugs.

The MDMA test kits containing the Marquis, Mecke, and Simon's reagents were purchased from DanceSafe and evaluated in a controlled laboratory setting utilizing three independent analysts. Thirty-nine tablets obtained for this portion of the study were street-grade Ecstasy tablets currently held as evidence in cleared cases from the Alachua County (Florida) Sheriff's Office and from the Forensic Toxicology Laboratory at the University of Florida. Using the instructions provided by DanceSafe, the Marquis reagent was judged alone and in combination with the Mecke and Simon's reagents. The identities of the tablets were confirmed by gas chromatography/mass spectrometry (GC/MS) operated in full-scan mode.

All three analysts generally agreed on the final identity of the tablets, although they did not agree on the colors observed. Two testers recorded four negative results, and one tester recorded 3 negative results, and 1 weakly positive result. Based upon GC/MS analysis, all 35 positive samples contained MDMA; however 8 were adulterated with other drugs including caffeine, ephedrine/pseudoephedrine, amphetamine, diazepam, and paramethoxymethamphetamine (PMMA). The four samples that tested negative were identified by GC/MS as alprazolam, ephedrine/pseudoephedrine with guaifenesin, ephedrine/pseudoephedrine with caffeine, and a tablet containing no identifiable drug (considered weakly positive by one tester).

Because the tablets held in evidence were known to contain controlled substances, and hence represented a biased selection, a follow-up evaluation was conducted. This part of the study consisted of two testers who were professionals knowledgeable in the field of toxicology, but inexperienced with the practical use of test kits. The testers were given the DanceSafe Test Kit and eight blinded samples. When tested, samples containing codeine, dextromethorphan, dihydrocodeine, ketamine, MDMA (2 each), morphine and d-norpropoxyphene produced many false positive and false negative results.

In addition to the disagreement between testers' conclusions and the inadequacy of the test results themselves, participants in all tests noted numerous problems with the kits. These problems included the inconsistency between the color charts provided in the instruction booklet and the actual colors observed during testing, the variation in the intensity of the color changes, and the variation in the rate of the reactions. It is also important to correlate these findings with the reality that these tests were designed for untrained personnel in an uncontrolled environment. Some potential issues include the lack of control samples provided for comparison, the lack of optimal lighting, the ambiguity in the written directions provided with the kits, the mental state of the user when reading the tests, the leakage of the cap seals after use, and the unpredictable drop times often leading to impatience and chemical spills. The latter issue creates an obvious danger from the reagents themselves because each one contains toxic and/or corrosive substance(s). Finally, accidental mixing of the reagents can be extremely hazardous.

In conclusion, these color tests are inadequate for use as harm reduction tools, especially in the hands of inexperienced users. If the goal of harm reduction is to reduce or minimize the risks associated with drug use, on no occasion in this study did the findings lead to avoidance of a contaminated drug. These tests have the potential to provide a false sense of security, encouraging the consumption of tablets whose composition is in question. There are potential consequences of ingesting a preparation containing a toxic ingredient, yet thought to be "pure" MDMA. Further, occasions occurred where the test reagents themselves caused injury to the tester or damage to the surroundings. While these types of tests may have a place in the hands of experienced personnel for forensic purposes, a decision on whether or not to ingest a tablet should not be made solely on the basis of these tests.

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Ecstasy, MDMA, DanceSafe Test Kit

K13 Oxycodone-Related Deaths in Delaware

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Attending this presentation will enable the participant to learn about: 1) the action of oxycodone; 2) a sensitive method for the analysis of oxycodone; and 3) the concentrations of oxycodone in postmortem specimens.

Oxycodone is a semisynthetic opioid analgesic derived from codeine that is indicated for the management of moderate to severe pain. Trade names for oxycodone containing preparations include Roxicodone, Percodan, Percocet, Roxicet, and Tylox. More recently, oxycodone has become available as a time release preparation (Oxycontin). The effects of oxycodone include euphoria, analgesia and sedation and it has a dependence liability similar to morphine. The typical adult dose (immediate release formulations) is 2.5-5 mg every 6 hours, although doses of 10-30 mg every four hours may be used for more severe pain. Extended release formulations are generally administered in doses of 10-80 mg every 12 hours. Therapeutic concentrations have been reported up to about 100 ng/mL.

The number of oxycodone-related deaths in Delaware has increased over the past 2 years. As a part of this study, blood and tissue specimens were analyzed for the oxycodone related deaths received from January 2001 through July 2002. Specimens were analyzed for oxycodone solid phase extraction (SPE) followed by gas chromatography-mass spectrometry. Briefly, the sample preparation procedure included deproteination, derivatization with hydroxylamine, SPE and derivatization

with BSTFA. Quantitation was performed using a 6-point calibration curve with d3-oxycodone as the internal standard. The limit of quantitation for oxycodone was 20 ng/mL. The heart blood, peripheral blood and liver oxycodone concentrations from 8 of these cases are summarized in the table below.

Case 1: A 29-year-old white female with a history of spinal fusion reported to the ER in a confused, emotionally labile state that progressed to somnolence and a coma. She received medical clearance from the ER to be transferred to a psychiatric hospital. She remained comatose and was found dead in her bed the next morning at the psychiatric facility. Thirteen "OC 40" pills were recovered from her gastric contents.

Case 2: A 57-year-old white female was found dead in bed at home. She had an extensive cardiac history.

Case 3: A 42-year-old white male was found unresponsive on the kitchen floor after an evening of heavy drinking at a bar the night before. He reportedly was offering "Oxycontin" tablets to other patrons at the bar.

Case 4: A 45-year-old white male was found dead on a couch. He had a history of alcohol and cocaine abuse. No cause of death was determined at autopsy.

Case 5: A 29-year-old white male was found dead in bed after an evening of playing games and drinking. His history included a renal transplant 2 years earlier, hypertension and diabetes mellitus.

Case 6: A 59 black female was found unresponsive in bed. She had reportedly not been feeling well for 3 weeks and refused to go to the hospital. She had a history of Hepatitis C, cirrhosis and mental status changes.

Case 7: A 50-year-oldwhite female complained of shortness of breath prior to collapsing and becoming unresponsive. She had a history of obesity and hypertension.

Case 8: A 39-year-oldwhite male who was found dead in bed. He had a history of chronic pain and had been diagnosed with Guillain-Barre neuropathy. His body was embalmed prior to examination.

The overlap between non-fatal and fatal oxycodone concentrations in the cases summarized above, as well as in additional oxycodone-related cases received at the State of Delaware OCME, has made interpretation somewhat complex. However, these data suggest that low concentrations of oxycodone in combination with alcohol and/or other drugs can cause death. In addition, the analysis of peripheral blood and tissue specimens demonstrated that postmortem redistribution did occur in some of the oxycodone-related cases.

Oxycodone, Postmortem, Distribution

K14 Evidence of Single Exposure to GHB Through Hair Analysis by GC/MS/MS

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The goal of this presentation is to demonstrate that by using segmental hair analysis and tandem GC/MS, it is possible to document a single exposure to GHB in a case of drug facilitated sexual assault.

Gamma-hydroxybutyric acid, or GHB is a substance naturally present within mammal species. Properties of neurotransmitter or neuromodulator are generally given to this substance. GHB is therapeutically used as an anaesthetic, but can be used for criminal offences (date-rape drug).

It appears that the window of detection of GHB is very short in both blood and urine, and therefore its presence very difficult to be documented after a rape case.

In order to document a single exposure, the interest of hair was investigated.

Hair was collected 1 month after the allegated event, in order to sample the corresponding period after regular growing.

After decontamination with dichloromethane, the hair shaft was cut into 3 mm segments. They were overnight incubated in 0.01N NaOH in presence of GHB-d_6, followed by neutralization and extraction in ethyl acetate under acidic conditions. GHB (parent ion m/z 233, daughter ions m/z 147 and 148) was tested by GC/MS/MS (Finnigan TSQ 700) after derivatization with BSTFA + 1% TMCS.

Responses for GHB were linear in the range 0.2 to 20 ng/mg. From 3 independent calibrations, the correlation coefficients ranged from 0.989 to 0.998.

The within-batch precisions were 11.8, 10.4 and 8.9 %, as determined by analyzing 8 replicates of 5 mg of hair obtained from the 3 subjects with GHB concentrations at 0.66, 1.30 and 2.45 ng/mg, respectively.

The extraction recovery (n = 3) was determined to be 81.8 %. The limit of detection of GHB was 0.1 ng/mg, using a 5 mg sample. This limit of detection can be improved by using a larger amount of hair. The limit of quantitation was the first point of the calibration curve, that is 0.2 ng/mg, below the endogenous levels.

Physiological concentrations (n=24) were in the range 0.5 to 12.0 ng/mg, with no influence with hair color.

Mean measured concentration were 2.21 +/- 0.57 and 2.47 +/- 0.69 ng/mg for males and females, respectively. The same results were obtained between hair samples of different colors (black, n=10 : 2.37 +/- 0.68 ng/mg; brown, n=6 : 2.21 +/- 0,71 ng/mg; blond, n=8 : 2.44 +/- 0.39 ng/mg).

No variation of concentrations was observed along the hair shaft in controlled subjects, excepted for the proximal segment, due to an incorporation through sweat.

A controlled human administration of 25 mg/kg to a volunteer demonstrated that a single exposure to GHB is detectable in hair after segmentation.

In a case of rape under influence, a clear increase of the corresponding segment (about 2.4 ng/mg) in time was observed, in comparison with the other segments (0.6-0.8 ng/mg). Hair color of the victim was brown and the result was not challenged by the rapist, who was arrested several days after the assault.

This study demonstrates that a single exposure to GHB in a case of sexual assault can be documented by hair analysis when collected about 1 month after the crime.

Fig: Hair segmentation in a case of GHB facilitated sexual assault
* : approximate time the assault occurred

GHB, Rape, Hair

K15 Rapid Screening of Psychotropic Drugs, Metabolites in Body Fluid and in Adulterated Liquor by Ion Mobility Spectrometry

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Rapid analysis and detection of diazepam and its metabolite in body fluids, which is of forensic importance, could be used in direct case/criminal examinations in forensic laboratories.

The misuse of psychotropic drugs by people is a common phenomenon. Diazepam is a drug belonging to the class of 1,4-Benzodiazepins. Its extensive use in therapeutics as a sedative, hypnotic, tranquilizer and muscle relaxant drug has also led to its misuse as a street tranquilizer and liquor adulterant. Owing to the synergetic action of diazepam when it is taken in combination with alcohol, it has recently come to replace chloral hydrate as an adulterant in alcoholic liquors. Diazepam is covered under the narcotic drugs and psychootroppic substances act (2), and its use in alcoholic liquors is prohibited. Samples of such abuse cases are frequently been submitted to labs for identification of tranquilizer with the aim to give correct treatment to the patient and law enforcement agencies to reach at the conclusion and putting the case to courts of law. The law enforcement agencies in the field are finding it difficult to identify diazepam and its over dosage in liquor adulterant cases on the spot and therefore have to necessarily send the exhibits of body fluids and the adulterated country liquor bottles on the spot and therefore have necessarily send the exhibits to forensic science laboratory for analysis. A wide literature survey was conducted on the field identification of diazepam. Diazepam can be detected using Cobalt (II) thiocynate acidified with orthophosphoric acid resulting the formation of intense emerald green colour. All this exercise requires rapid, sensitive and accurate analysis by the analyst.

Keeping this in view, the authors have developed and described the method based on "Ion Mobility Spectrometry" to detect diazepam and its major metabolite Desmethyl diazepam (nordiazepam) which is present in liver, kidney of viscera of the dead body. The Used urgent need for the development of simple, presumptive field and screening techniques useful for law enforcement officers and mobile forensic science laboratories, for the detection of diazepam and its metabolite. The technique "Ion Mobility Spectrometry" has been developed and detected in the form of Plasmagram which was reported in this communication. It can be used for the identification of various drugs such as Cocaine, Herion, methaqulone, etc., as well.

Tranquilizer, Ion Mobility Spectrometry, Diazepam

K16 A Rapid Method for the Determination of Benzodiazepines in Postmortem Blood by HPLC-MS-MS

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Attendance at this presentation will enable the participant to learn about a new method for the qualitative determination of benzodiazepines in postmortem blood specimens by HPLC-MS-MS. This method is a comprehensive screen for these drugs and it is rapid, robust and reproducible over a wide range of concentrations.

The Forensic Toxicology Service offers a National screening and quantification toxicology service to Coroners and Forensic Pathologists, as well as to Police Forces. As a result, screening for benzodiazepines is required in a large number of postmortem specimens followed by quantification of those detected. Benzodiazepines remain the U.K.'s most commonly abused prescription drugs. Examples include diazepam (Valium), lorazepam (Ativan), nitrazepam (Mogadon - "moggies") and temazepam ("jellies," "egg"). In order to be able to offer a more rapid, reliable and robust method for their identification and quantification, a method for heir analysis by HPLC-MS-MS using a minimal amount of postmortem blood has been developed.

The analytes of interest are extracted from postmortem blood as follows: in a 2mL polypropylene tube 100µL of the blood specimen is added, 250µL of phosphate buffer (pH 7.0), 100µL of prazepam (1mg/L) as internal standard and 1mL methyl tert-butyl ether (MTBE). The contents are then mechanically mixed for 5 minutes and centrifuged for 1 minute at 12000 rpm. The top, organic, layer is then transferred to a clean 4.5mL polypropylene tube and evaporated to dryness using a Savant SpeedVac SC200 coupled to a Savant RT 4104 refrigerated condensation trap. The residue is reconstituted in 250µL of 80% methanol, vortex mixed for 20 seconds and transferred to a polypropylene auto-sampler vial. 10µL of the sample is then analyzed by HPLC-MS-MS. The total run time on the HPLC-MS-MS for each specimen is less than 4 minutes.

The analytical column used is a 15cm x 4.6mm (id) Supercosil LC-18-DB (5µm particle size) ODS column maintained at 50ºC using a Perkin Elmer series 200 column oven. Isocratic solvent delivery is achieved using a Perkin Elmer series 200 pump set at 1 mL/min. Sample injection, 10µL, is performed by a Perkin Elmer series 200 auto-injector. The mobile phase consists of methanol/water (85:15, by volume) supplemented with ammonium acetate solution to achieve a final concentration of 2 mmol/L.

Detection is by tandem mass spectrometry (HPLC-MS-MS), using a Sciex API2000 triple quadrupole mass spectrometer (Applied Biosystems). A turbo ion spray (heated electrospray) source heated to 300°C is used to introduce the sample into the mass spectrometer. A post-column splitter (10:1) is installed just before the ion spray interface. The mass spectrometer is operated in positive ionization, multiple reaction mode (MRM, MS-MS), with the resolution set to unit resolution (±0.5m/z). High purity air is used as the nebulizer gas and high purity nitrogen as the collision gas.

The Applied Biosystems Sciex Analyst software is used to control the HPLC-MS-MS, record the output from the detector, integration of peak areas and calculation of peak area. In assays requiring quantification, the Analyst software is used to calculate the peak area ratios, produce the calibration line using $1/x^2$ weighed through zero regression and to calculate the concentration of each analyte.

A reference mixture of control substances made in drug-free human blood is extracted and run with all unknown samples as part of every assay. These reference concentrations were selected as they approximate the lower levels of the respective substance in blood following their therapeutic intake, as noted from the literature. The table below presents

compounds contained in the current reference mixture, their respective final concentrations, together with the target masses used for their identification.

Drug Name	Concentration (mg/L)	Q1 m/z	Q3 m/z
Alprazolam	0.05	308.9	280.9
Chlordiazepoxide	0.50	299.9	227.1
Chlordiazepoxide Lactam	0.30	286.8	180.1
Citalopram	0.05	325.2	109.1
Clobazam	0.10	300.9	259.0
Desmethylclobazam	0.20	286.9	245.0
Desmethyldiazepam	0.20	271.0	165.1
Diazepam	0.10	284.9	153.9
Lormetazepam	0.05	345.0	289.2
Lorazepam	0.05	320.8	275.0
Loprazolam	0.05	465.2	111.3
Nitrazepam	0.05	281.9	236.0
Oxazepam	0.10	287.0	156.1
Prazepam (Internal Standard)	N/A	325.1	271.1
Temazepam	0.40	300.9	255.0

The method has performed satisfactorily for samples from cases involving either therapeutic use or overdose with these agent. Due to the high sensitivity of the instrument used, acute overdoses require reconstitution in a larger volume of solvent prior to injection. The limit of detection for the chosen analytes was better than 1/10th of the respective lower therapeutic level as analyzed in the reference mixture. The limits of detection and quantification can be improved by increasing the injection volume, from 10µL to 50 or even 100µL, if required. Following the general screen by the method described herein, the remaining extracts are analyzed by a different HPLC-MS-MS method for benzodiazepines with lower concentration ranges such as flunitrazepam.

The method described here is rapid (total run time on the HPLC-MS-MS is less than 4 minutes for each specimen), reproducible and robust and can be applied in forensic toxicology laboratories for the screening of benzodiazepines, a family of sedative hypnotic drugs commonly used and abused worldwide.

Forensic Toxicology, Benzodiazepines, HPLC-MS-MS

K17 Screening Postmortem Whole Blood for Oxycodone by ELISA Response Ratios

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The goal of this presentation is to demonstrate screening of postmortem whole blood for oxycodone using the ratio of the oxycodone immunoassay response to the response for the specimen obtained with an opiate immunoassay.

A number of cases of diversion of OxyContin® and related prescription opiate narcotics for illegal use and abuse have been in the national press this past year. As a result of the popularity of these drugs, oxycodone may be increasingly encountered in driving, abuse and overdose cases. Oxycodone and related semisynthetic thebain derivatives may be missed by general opiate screens which are weakly cross-reactive with the C6-oxy opiates and by confirmation procedures which use GC/MS Selected Ion Monitoring (SIM) parameters set for morphine and codeine. Immunoassay response ratios can be used to identify which opiate positive specimens may contain oxycodone or related opiates. By dividing the response of a second oxycodone-directed immunoassay by the specimen response in a general opiate screen immunoassay, a relative immunoassay response ratio is obtained. Oxycodone-involved cases can be indicated by response ratios above an empirical cutoff threshold. This elevated ratio indicates which specimens should be confirmed for oxycodone, oxymorphone, hydrocodone and/or hydromorphone in addition to the confirmation for morphine and codeine.

Forty-eight specimens, which were negative for opiates, and one hundred sixty seven postmortem whole blood specimens, which were positive for opiates, including sixty-six specimens known to contain oxycodone, were assayed. Specimens were diluted 1:5 with assay buffer and analyzed by both the Neogen Oxymorphone/Oxycodone ELISA and the Neogen Opiate Group ELISA (Neogen Corporation, Lexington KY). Both immunoassays are microtiter plate-based ELISAs using horseradish peroxidase-labeled drug and anti-drug antibody immobilized to the microplate wells. Spiked whole blood calibration standards, specimens, the manufacturer's EIA standard, and negative and positive synthetic urine based controls were run on each plate. For the Opiates Group ELISA, standard concentrations were 0, 1, 5, 10, 20, 50 and 100 ng/ml morphine. For Oxymorphone/Oxycodone ELISA, the spiked standard concentrations were 0, 1, 5, 10, 20, 50 and 100 ng/ml oxymorphone. Diluted drug-enzyme conjugate was added to the microtiter plate wells and the mixture incubated at room temperature for 45 minutes. After incubation the plate was washed five times with wash buffer (phosphate buffer with Tween 20) using a Bio-Tek Elx50 Microplate Strip Washer (Bio-Tek Instruments, Highland park, Winoski, VT) to remove any unbound sample or drug-enzyme conjugate. K-Blue® substrate (tetramethylbenzidine (TMB) plus hydrogen peroxide) was added and after a 30-minute substrate incubation, the reaction was halted with the addition of Red Stop Solution (a non-acid peroxidase stop solution). The test was read using an Elx800 Universal Microplate Reader equipped with a 650 nm filter (Bio-Tek Instruments, Highland Park, Winoski, VT).

Calibration curves were plotted as log concentration vs the logit of the ratio of the mean absorbance at each concentration divided by the mean absorbance of the zero standard (B/B_o). The oxymorphone or morphine equivalents were estimated from the calibration curve using the ratio of the mean absorbance of the specimen to the mean absorbance of the zero standard.

The oxymorphone equivalents in ng/ml from the Oxymorphone/Oxycodone ELISA were divided by the morphine equivalents in ng/ml from the Opiates ELISA to obtain an Oxycodone/Opiates Response Ratio. This ratio was compared to the GC/MS data for all specimens and for opiate positive specimens.

Sensitivity, the true positive rate, was calculated from the tally of true positives and false negatives determined by comparison of the GC/MS findings as: Sensitivity = TP/(TP + FN). Specificity was calculated as: Sensitivity = TN/(TN + FP). Because sensitivity and specificity are probabilities, the standard error (SE*p*) is equal to SE*p* = square root [p(1-p)/n]. Receiver Operating Curves (ROC) were obtained by plotting the sensitivity at each putative response ratio cutoff vs. (1 - specificity) at that cutoff value. The positive predictive value was calculated as fp/[fp + (1-f)(1-q)] where f is the prevalence in the population to be tested, p is the sensitivity and q is the specificity.

Specimens containing oxycodone produced large responses in the Oxycodone-directed immunoassay and positive but weaker responses in the general Opiate Group immunoassay. The median response ratio for oxycodone-containing specimens was 12.9; the mean was 33.7. The

median response ratio for all opiate positive specimens **not** containing oxycodone was 0.055 and the mean was 7.3. ROC analysis was used to find an optimum response ratio cutoff value and to determine the probability that a specimen with this ratio would contain oxycodone or a related C6-oxy opiate. The optimum relative response ratio was 2.0. Specimens with a relative response ratio of 2.0 or higher had a greater than 50% probability (positive predictive value) of containing oxycodone. The sensitivity of the ELISA response ratio for the presence of oxycodone at a response ratio cutoff of 2.0 was 89.4% ± 3.8% and the specificity was 88.1% ± 3.2%.

The Neogen Opiates Group ELISA has a crossreactivity of 730% for codeine relative to 100% for morphine, 228% for hydrocodone, 35.6% for hydromorphine, 5.2% for oxycodone and 0.22% for oxymorphone. The Neogen Oxymorphone/Oxycodone ELISA has a cross-reactivity of 400% for oxycodone and 100% for oxymorphone, 30.8% for hydrocodone and 12.3% for hydromorphone; the crossreactivity with codeine is only 5.3% and for morphine 1.7%. The oxymorphone/oxycodone immunoassay has sufficient selectivity to identify OxyContin®- and other oxycodone-involved cases by using the ratio of the relative response to the Neogen Opiate Group ELISA result. Neither assay had a response within the calibration curve range with the negative whole blood specimens. However, some decomposed specimens caused false positive results with the ELISA assays. In conclusion, the Neogen Oxymorphone/Oxycodone ELISA can be used as a second immunoassay to identify which opiate-positive specimens should be confirmed for oxycodone.

Oxycodone, ELISA, Response Ratio

K18 Ultra-Fast Determination of Metformin in Plasma by Hydrophilic Interaction Chromatography: Application in a Fatal Case of Metformin Self-Poisoning

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The learning objective of this paper is to present an ultra-fast and accurate method for the determination of metformin in plasma by hydrophilic interaction chromatography on a special stationary phase. This paper will present the toxicological results, together with the clinical data in a fatal case of lactic acidosis due to a self-poisoning by metformin. The authors will demonstrate that assay of this biguanide in plasma may contribute towards the differential diagnosis of the acidosis.

Metformin (1,1-dimethylbiguanide, hydrochloride or p-chlorophenoxyacetate salt) is an oral hypoglycemic agent, widely used in France and Europe in the treatment of type II non-insulin dependent diabetes mellitus. This medication is considered safe if not used in the presence of contraindications. Daily oral doses range from 200-1500 mg as metformine base and even at doses higher than 85 g at once, no hypoglycemia was observed. The main adverse effect at high dose is an extremely severe lactic acidosis. Describe here a fatal case of metformin self-poisoning with a severe lactic acidosis and a special ultra-fast analytical method by HydrophILic Interaction Chromatography (HILIC) with photodiode array detection.

Case report: A 42-year-old Caucasian male was admitted to hospital for confusion and acute abdominal pain. He has a ten-years history of non-insulin dependent diabetes mellitus and received metformin therapy. However because of inadequate glycemic control under biguanide in this mentally deficient patient, metformin was stopped one year ago and insulin therapy was used, together with ibuprofen and tiaprofenic acid. At the admission, the patient was conscious but confuse and he argued attempting suicide with an unknown amount of metformin. He suffered of oliguria, important dehydratation, hypothermia, hypotension, renal failure and lactic acidosis with normal glucose (pH 6.88, bicarbonate 2.9 mmol/L, lactate 27 mmol/L, creatinine 163 µmol/L, glucose 16.4 mmol/L). The treatment consisted in a mechanical respiratory support, correction of fluid deficits, treatment of hypothermia and correction of acidosis by hyperventilation. Despite these intensive care, the patient developed an acute respiratory distress syndrome, anuria and shock. He died 34 hours after admission. A blood sample was obtained just before death.

Analytical conditions : Metformin is a very little polar molecule and so is very difficult to extract by classical organic solvents and to assay by reverse phase liquid chromatography (HPLC) because of a short retention time on octyl or octadecyl hydrophobic phases. HILIC was chosen to perform a fast and accurate method to determine metformin in plasma, because this method is well suited for the separation of little polar molecules. The HILIC column (200 x 4.6 mm) contains a poly(2-hydroxyethylaspartamide)-silica stationary phase (PolyHydoxy Ethyl A, PolyLC, USA)with 5µm particules. Mobile phase was acetonitrile : phosphoric acid (65 ; 35, v/v) at pH 2.8, with a flow-rate at 1.5 mL/min. Metformin was detected by a diode array detector at 234.6 nm (Waters 996). Extraction of metformin from plasma was very easy and fast, just adding 15 µL diluted perchloric acid to 250 µL plasma in order to precipitate the proteins.

Results and discussion: The mean retention time of metformin is 2.90 +/- 0.24 min. The linearity of the method is very good from 0.1 to 400 µg/mL (r^2 = 0.99). The limit of detection is 0.02 µg/mL, the limit of quantification is 0.1 µg/mL. Recovery from plasma is excellent, reaching 99.5%. The authors verified that there is no analytical interference between metformin and other oral hypoglycemic medications (glibenclamide, glicazide, glipizide and benfluorex) : they are all eluted in the solvent front between 1.1 and 1.3 min.. Intra-day and inter-day variabilities are higher at low concentrations (cv = 20% at 0.25 and 1 µg/mL) than at higher concentrations (cv = 2% at 50, 200 and 400 µg/mL). Plasma metformine concentration was 188 µg/mL. International literature syas that therapeutic blood concentrations range from 0.75-3 µg/mL and toxic concentrations from 5-250 µg/mL. However, the prognosis of metformin poisoning mainly depends on the concurrent pathology and administration of other medications: that is what was observed in this patient (dehydratation and two non-steroidal antiinflammatory drugs

Conclusion: The best HPLC analytical solution for the determination of polar xenobiotics is to use polar stationary phase eluted with an aqueous-organic mobile phase (HILIC). This technique appears to be well suited to determine metformin in plasma and it was applied in a fatal case of metformin intoxication. The developed method is ultra- fast (less than 20 min) and accurate. As far as the symptoms of biguanide poisoning are non-specific, diagnosis of metformin intoxication may be improved by a rapid determination of the drug in blood.

Metformin, Hydrophilic Interaction Chromatography, Poisoning

K19 Analysis of Barbiturates by Fast GC: A Preliminary Study

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The goal of this paper is to present information on an evaluation of a fast gas chromatographic method developed for the analysis of barbiturates.

Fast gas chromatography (GC) has the potential to be a very useful tool in toxicological analysis by shortening retention times and increasing the overall rate of analysis. The most common techniques for achieving fast GC analysis include shortening of the GC column, raising the GC oven temperature, and increasing the GC oven ramping parameters. With these simple techniques in place, drugs can be analyzed more efficiently.

In the present study, a fast GC method was developed for the analysis of amobarbital, butalbital, pentobarbital, phenobarbital and secobarbital. The barbiturates were isolated from 1.0 mL of whole blood using CleanScreen® solid-phase extraction (SPE) cartridges (ZSDAU020) manufactured by United Chemical Technologies, Inc. Following elution from the SPE cartridge, the extracts were dried under a gentle stream of nitrogen at 40°C and reconstituted in a dilute methanolic solution (0.02 M) of trimethylanilinium hydroxide.

The extracts were analyzed using a Hewlett-Packard 6890 Series gas chromatograph equipped with a nitrogen-phosphorus detector. The inlet and detector temperatures were set at 250°C and 330°C, respectively. Helium was used as the carrier gas at a flow rate of 0.1 mL/min. Automated injections of 0.5 mL, at a split ratio of 31:1, were made onto an Agilent Technologies DB-5 (10 m x 100 mm x 0.1 mm) GC column. The initial oven temperature of 120°C was held for 0.25 min., then ramped 30°C/min. to a final temperature of 320°C for 0.75 min. The total run time was 7.67 min. These oven parameters were achieved by reducing the internal GC oven volume with the aid of an oven insert, as well increasing the GC power supply voltage to 220 V, from the standard 120 V.

A five-point calibration curve was prepared in drug-free whole blood in a range of 2.5 mg/L to 25 mg/L. Quantification was performed with barbital as the internal standard fortified at a concentration of 10 mg/L. In order to assess the intra- and inter-run accuracy and precision of the assay, control samples were prepared at 7.5 mg/L and 12.5 mg/L and assayed five-times each in three separate experiments. Finally, a correlative study utilizing specimens previously assayed by a conventional GC method was conducted.

Under the fast GC conditions described, all barbiturates eluted from the GC column within 5 min.; the total GC cycle time was 9-10 min. This increase in throughput had no effect on chromatographic performance and analyte resolution. The results of the validation studies demonstrated excellent accuracy and precision with %CV values in the range of 15% or less and % accuracy values in the range of 90% or greater. Further, correlation was good between the conventional GC and fast GC methods.

While the fast GC method has distinct advantages, mainly improved efficiency, some limitations do exist. First, poor resolution between some analytes was evident. For example, under the conditions described, butalbital and butabarbital, and hexobarbital and caffeine, _co-eluted. Similarly, high concentrations of caffeine interfered with the quantitation of phenobarbital. Another potential limitation of the fast GC method is the decreased capacity of a narrow bore GC column which may lead to column overload and reduced range of analyte linearity.

In conclusion, fast GC has great potential to become an efficient method for routine toxicological procedures in forensic toxicology laboratories. Because this method reduces the GC cycle time by nearly two-fold, it significantly increases laboratory throughput.

Fast Gas Chromatography, Barbiturates, Solid-Phase Extraction

K20 A Tale of Two Drugs in Southwestern Virginia: Oxycodone and Methadone

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The objective of this presentation is to provide forensic toxicologists and pathologists with statistical drug-related case data for a five year period from 1997 to 2001 for the drugs oxycodone and methadone. To understand patterns of oxycodone and methadone misuse and abuse and become familiar with factors responsible for absolute and relative changes in case statistical profiles over time.

The problem of prescription drug misuse and abuse contributes to significant morbidity and mortality in Southwestern Virginia. Opiate and opioid drugs are in great demand by misusers and abusers. Beginning in 1999 the Toxicology Section of the Virginia Division of Forensic Science (DFS) Western Laboratory together with the Office of the Chief Medical Examiner (OCME) for the Western Region noted a dramatic rise in drug-related fatalities involving oxycodone. The trend continued through 2000 and 2001. Investigative information, drug paraphernalia recovered from death scenes, decedent history and witness statements chronicled in a substantial number of cases implicated involvement of the sustained release formulation of oxycodone (OxyContin®). OxyContin®, a single-drug entity designed for sustained release over a 12 hour period, is easily compromised by abusers to achieve a powerful morphine-like high. The drug is diverted and supplied to abusers by a number of means: Illegal prescriptions by unscrupulous physicians ("pill mills"), illicit black market sales, pharmacy thefts, fraudulent prescriptions, "doctor shopping," and diversion from sources in Mexico and Canada.

Methadone, a drug traditionally used as a heroin substitute for treating addiction, exhibited a similar increase in frequency in Western District postmortem cases over the same time period. Methadone is also prescribed in the treatment of chronic pain syndromes. The data suggests a hypothesis of a classic "supply and demand" scenario. Intense interdictive efforts by law enforcement, attention by legislative officials and widespread media attention curbed "supply" of oxycodone, but not "demand." Additionally, physicians cognizant of the controversy substitute methadone for the treatment of chronic pain syndromes formerly managed with oxycodone, more specifically, OxyContin®.

The data presented includes: the total number of Western Region drug-related deaths, cases in which oxycodone and methadone were determined to be significant in terms of cause of death and statistics in which oxycodone and methadone were determined to be present in the blood and postmortem tissues of decedents. Retrospective review of information in the DFS database and information derived from the database of the OCME for the Commonwealth of Virginia constituted the methodology of the study. OCME, Western Region certified 519 drug deaths from 1997 to 2001. Thirty-four percent (n=175) of the certifications identified oxycodone (n=82) or methadone (n=93) as being significant to the cause of death. The three-year period 1999 to 2001 illustrated increases in the total number of drug deaths and deaths attributed to oxycodone and methadone. Deaths attributable to oxycodone or methadone represented the following proportions for the period 1999-2001: Thirty-one per cent, thirty per cent and fifty-eight per cent, respectively. OCME, Western Region conducted 676 autopsies in the most recent calendar year (2001), certifying 23 per cent as drug-related (n=155). Fifty-eight per cent (n=90) identified oxycodone or methadone as primary agents in establishing cause of death. All cases

were initially screened by FPIA (Abbott TDx™) and a basic drug screen using solid phase extraction (SPE) followed by GC-NPD and/or GC-MS. Methadone was quantitated using a basic drug SPE and GC-NPD or SIM GC-MS. Oxycodone was quantitated by forming oxime/TMS derivatives and SIM GC-MS. Mean blood methadone concentrations during the 1997-2001 period were 0.28 mg/L (n=6), 0.29 mg/L (n=8), 0.36 mg/L (n=19), 0.47 mg/L (n=22) and 0.65 mg/L (n=50), respectively. Mean blood oxycodone concentrations during the 1999-2001 period were: 0.61 mg/L (n=21), 0.37 mg/L (n=27) and 0.62 mg/L (n=39), respectively.

Conclusions from the data indicate serious public health concerns posed by the misuse and abuse of prescription drugs, particularly opiate and opioid drugs such as oxycodone (OxyContin®) and methadone. Southwestern Virginia's experience in this regard is similar to reports of abuse in other geographical regions of the U.S. An initial trend in the misuse and abuse of oxycodone, principally in the form of OxyContin®, followed by alarmingly widespread misuse and abuse of methadone resulting in remarkable drug-related mortality. The potential or likelihood of abusers seeking other opiate and opioid drugs (e.g., heroin, hydrocodone, fentanyl and hydromorphone) when "supply" of oxycodone and methadone is curtailed is a disturbingly high probability.

Oxycodone, Methadone, Abuse

K21 Evaluation of Ephedrine, Pseudoephedrine, and Phenylpropanolamine Concentrations in Human Urine Samples and a Comparison of the Specificity of, DRI® Methamphetamine and Abuscreen® Online Abuscreenâ Online Screening Immunoassays

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The purpose of this study was to evaluate the ability of two amphetamine-like screening reagents to exclude ephedrine (EPH), pseudo ephedrine (PSEPHE), and phenylpropanolamine (PPA) from producing false positive screening results. The study also sought to characterize the prevalence and concentration distributions of human urine samples containing EPH, PSEPHE, and PPA that produced positive screening results for the amphetamine drug class.

Two immunoassays were evaluated DRI® amphetamines and Abuscreen® Online amphetamines. Reagents were run according to manufacturer specifications using a Hitachi Modular DDP system. Approximately 27,400 randomly collected human urine samples from Navy and Marine members were screened. All assays were calibrated using a single point, qualitative cutoff standard with the manufacturer recommended compound at the department of defense cutoff (500 ng/ml). Samples were prepared by solid phase extraction after the pretreatment with sodium periodate and addition of d11 AMP, d14 MTH, d5 MDA and d5 MDMA as internal standards for the determination of AMP, MTH, MDA and MDMA. For the determination PSEPH, EPH, and PPA (in samples which did not confirm for the presence of AMP, MTH, MDMA or MDA) a similar solid phase extraction was utilize with out pretreatment with sodium periodate and with n-ethyl-benzylamine used as the internal standard. GC/MS was used for the analysis of all samples as previously described [stout et al JAT 2002 26:XX-XX]

As previously reported, one thousand one hundred and four samples screened positive by the DRI AMP kit of which 1.99% confirmed positive for the presence of AMP, MTH, MDA or MDMA. For the Online reagent 317 screened positive of which 7.94% confirmed positive for AMP, MTH, MDA or MDMA. Eight hundred and thirty three of the non-confirming samples were confirmed for the presence of EPH, PSEPH and PPA, all contained PSEPH. The mean PSEPH concentration was 126,000 ng/ml with a range of 5,700 ng/ml to 2,500,000 ng/ml. Consistent with the relative reported cross reactivities, concentrations of samples positive by DRI only were less than those positive by both Online and DRI (DRI mean for 574 samples of 84,000 ng/ml and for 258 Online positives of 218,000 ng/ml $p<0.05$). PPA was present in 28% of the samples with a mean concentration of 18,000 ng/ml and a range of 285 ng/ml to 448,000 ng/ml. EPH was present in 30% of the samples with a mean concentration of 64,000 ng/ml and a range of 437 to 859,000 ng/ml. In 76% of samples where PPA was present it was present in concentrations greater than 10% of the PSEPH concentration. In all but 4 samples (2%), when PPA was present, so was EPH. When compared to the entire screened sample set, PSEPH was present in approximately 3%, EPH in 0.9% and PPA in 0.8% of the samples.

The results indicate that cross reactivities for EPH, PSEPH and PPA are greater than reported for these reagents. While the reagents may produce fewer false positives due to PSEPH at a cutoff of 1000 ng/ml, at a 500 ng/ml cutoff a substantial number of false positive screening results were obtained. This indicates that continued work is necessary to improve the specificity of amphetamine screening reagents particularly if lower cutoff concentrations are to be used.

The distribution of concentrations indicates that very large concentrations of EPH, PSEPH and PPA are common. The presence of PPA was striking in its prevalence in light of the removal of PPA containing over the counter products. Also PPA was present in concentrations far in excess of what would be expected from reported metabolism of PSEPH to PPA (approximately 1%). This suggests either a continued commercial source of PPA containing products or PPA as a possible substantial contaminant of some EPH and PSEPH containing products.

Immunoassay, Ephedrine, Pseudoephedrine

K22 Elimination of Ketamine and Norketamine in Urine of Nonhuman Primates After a Single Dose of Ketamine

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Upon completion of this presentation, the attendee will understand the principles of extraction and detection of ketamine (KET) and norketamine (NKET) in urine using NCI-GC-MS, 2) concentrations of KET and NKET in nonhuman primate urine after a single dose of the drug.

The general anesthetic ketamine (Ketalar®, Ketaject, Vetalar) (KET) is used in human and veterinary medicine for induction of anesthesia for short surgical procedures and routine veterinary procedures. It has also been identified as a so-called "date-rape" drug for the purpose of "drugging" unsuspected victims and raping them while under the influence of the drug. Its illicit use by teenagers in rave parties has also been reported. The objective of this paper was to study elimination of KET and its major metabolite norketamine (NKET) in urine collected from five nonhuman primates which received a single dose of KET, and to study elimination patterns to determine how long after drug administration, KET and metabolite can be detected. The data are of great

importance to law enforcement agencies and the forensic toxicology community in order to determine how long after sexual assault the urine samples can be collected from the victim to successfully prosecute the perpetrator. The aim of this study was: 1) to develop and validate highly sensitive NCI-GC-MS method for the simultaneous quantitation of KET and its major metabolite NKET in urine, 2) to analyze urine samples collected from nonhuman primates which received a single dose of KET, for NKET and KET.

Method: Urine was collected from five stumptail macaques (*Macaca arctoides*), four females (8-19 kg) and one male (17 kg) caged individually. All animals received a wash-out period of six months prior to the experiment. One urine sample was collected from each animal before KET administration. All monkeys received a single dose (5 mg/kg, IM) of KET. Urine samples were collected from each animal for 18 hours every day (excluding weekends) up to 24 days and once every four days up to 35 days.

Extraction: All urine samples (2 ml) were extracted from urine using HCX solid phase extraction columns. Five point standard curves for KET and NKET were prepared by spiking aliquots (2 ml) of negative urine. The range of the standard curves was 20-1,000 ng/ml for KET and 50-5,000 pg/ml for NKET. In addition, two levels of control urine preparations were analyzed (100 pg/ml and 1,200 pg/ml for NKET, and 40 ng/ml and 750 ng/ml for KET). To all standard, control and study samples, internal standards (D_4 NKET 1,000 pg/ml), 0.1 M acetate buffer (pH 4.5, 1 ml) and crude β-glucuronidase solution (50 ml) were added, and samples were incubated for 1.5 hours at 37°C. After incubation 1.93 M acetic acid (1 ml) and deionized water (10 ml) were added. An analytical column was conditioned with methanol (3 ml) deionized water (3 ml) and 1.93 M acetic acid (1 ml), the sample was added and the column was washed with deionized water (3 ml), 0.1 N HCl (1 ml) and methanol (3 ml). The final elution from the extraction column was achieved using methylene chloride:isopropanol:ammonia (78:20:2:, v/v/v, 3 ml). All extracts were evaporated to dryness in the stream of nitrogen, dissolved in ethyl acetate (50 ml) and transferred to autosampler vials. Dried samples were derivatized (30 min, 60°C) using HFBA (50 ml). HFBA was evaporated under vacuum and the dry residue was dissolved in ethyl acetate (25 ml).

Analytical Procedure: A Hewlett-Packard GC-MS instrument (6890 GC and 5973 MSD) operating in chemical ionization mode was used for the analysis. The column was an HP5-MS (30 m length x 0.2 mm i.d. x 0.25 mm film thickness) and the collision gas was methane maintained at an ion gauge pressure of 3.9×10^{-4} Torr. The injector temperature was 240°C, the transfer line was 280°C and the source and quadrupole were kept at 200°C and 106°C, respectively. The oven was held at 60°C for 1 min then ramped at 30°C/min to a final temperature of 310° where it was held for 3 min. The injection volume was 1 ml. The monitored ions for KET derivative were *m/z* 226 and 357, for NKET *m/z* 383 and 399, and for D_4 NKET *m/z* 387 and 403.

Results: In two monkeys KET was detected in urine up to three days after drug administration (7,070-32 ng/ml), in one up to four days (13,500-65 ng/ml), in one only on day 1 and 2 (4,000 and 70 ng/ml, respectively), and in one animal ten days after KET injection (35,000-22 ng/ml). NKET concentrations in urine ranged from 1.75 mg/ml to 63 pg/ml and it remained in urine throughout the entire 35-day study period in four out of five animals. In one monkey NKET was detected up to 31 days after KET administration.

Date-Rape Drugs, Ketamine and Norketamine, Urine, NCI-GC-MS

K23 Impaired Drivers in the Canton Bern (Switzerland) With Benzodiazepine Detections

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The learning objective of this presentation is to demonstrate that several benzodiazepines alone are causing a safety problem on the roads of Bern in comparison to people who overuse/abuse alcohol or illicit drugs.

The vast majority of DUI problems continue to come from the overuse/abuse of alcohol alone. Cannabis alone, or combined with alcohol, is the second commonest problem and it is growing. Misusers/abusers of opiates, cocaine, and amphetamines constitute the next group. This laboratory many DUI of drug cases in which benzodiazepines alone are causing driving impairment.

Methods: in this part of Switzerland protocols and procedures have been developed to aid the police initially to recognize the possibility that a person is DUI of drugs (Police report of suspected inability to drive safely) and then, to initiate a chain of events to ensure that secure forensic evidence is acquired. For DUI of drug cases the next step is the medical examination. The MD takes two blood and one urine sample and fills in the report of medical examination. In the laboratory the urine screening is performed by EMIT or in special cases by GC-MS. Alcohol is determined in the blood sample by head-space GC-FID. The quantitative determination of drugs in the blood samples is performed by GC-ECD (benzodiazepines), GC/MS (opiates, THC, THC - COOH, cocaine, EME, BE), GC-NPD, or HPLC-DAD (basic drugs). The results are reported to the legal authorities.

Using DUI cases to provide a "snapshot" of the more general drug abuse scene drug misuse/abuse is often a matter of street "fashion" with drugs of misuse changing according to whim. This is particularly true of the benzodiazepines, which are seldom misused alone. Rather they are almost invariably misused by people who misuse/abuse other drugs or alcohol. DUI cases of people who abuse benzodiazepines in combination with heroin or methadone to survive from one opiate dose to the next is observed; Cocaine or amphetamine users to get down from their high; "Self-medicators" – they use benzodiazepines to calm their anxieties. The benzodiazepines may or may not be legitimately prescribed. "Self-medicators" may combine benzodiazepines with alcohol, despite specific warnings not to do so.

DUI, Benzodiazepines, Switzerland

K24 Liquid Codeine: New Drug, Same Old Song

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The goals of this presentation are to increase the awareness of the presence and high abuse statistics of liquid codeine, e.g., cough syrup, and to emphasize the need for laboratories and law enforcement agencies to analyze for this drug and track its abuse, respectively.

There has been a significant rise in the manipulation of liquid codeine from its licit pharmaceutical use to a new drug of abuse. Its abuse has received great popularity in recent years for young minorities in the Harris County area. Songs have been written encouraging such

practices. The trend is most evident among African-American males. the laboratory has noted this trend based upon physical evidence and biological samples received from various law enforcement agencies and forensic pathologists, respectively.

The liquid codeine submitted to the Controlled Substance Laboratory has increased approximately two hundred fifty percent in both 2000 and 2001 as compared to the total for 1999. Liquid exhibits are received as bulk pharmaceutical containers, individual prescription bottles, or diluted in a variety of soda flavors. Over 90% of the typical abusers or possessors are African-American males who range in age from 16-40 years old.

In addition to this increase of confiscated drugs, the Toxicology Laboratory has witnessed an increased presence of codeine with promethazine in specimens submitted for suspected driving under the influence (DUI) and death investigation cases. Three deaths are described and two alleged DUI cases presented to the Medical Examiners Office in Harris County, TX within eight months of time.

The first postmortem case is a 29-year-old African-American male who was found dead in his recording studio. According to friends, he had been drinking cough syrup and possibly using other illegal drugs. The decedent was last seen alive in the early morning hours when everyone fell asleep. Toxicological analysis revealed codeine present at 1.74 mg/L in the blood. Phencyclidine was also present in the blood and urine. The cause of death was ruled a codeine overdose with mixed drug intoxication.

In the second case, a 61-year-old white female was found dead in her home by her daughter. She had made prior suicide attempts and was reportedly depressed. A large bottle of alcohol was found half empty near her body. Toxicological analysis of blood found toxic levels of codeine present at greater than 20.0 mg/L. The cause of death was ruled as codeine toxicity.

In the third case, a 16-year-old Hispanic male was reportedly playing "Russian Roulette" at a party at a friend's house and shot himself with a revolver he had displayed earlier. Friends asserted that he appeared to have been drinking before he arrived. Toxicological analysis revealed codeine present at 1.29 mg/L in the blood. Ethanol was also detected in the blood, urine and vitreous humor.

The abuse of codeine has also risen in driving under the influence cases. In the first case, a 19-year-old white male was stopped for a routine traffic violation. The officer detected an odor of marijuana. He also noticed the defendant to be unsteady, having red eyes and slurred speech. Laboratory analysis of urine identified codeine, promethazine, marijuana metabolite and alprazolam.

In the second DUI case, a 22-year-old black male was seen driving a badly wrecked vehicle when an officer tried to pull him over. He was evading arrest and committed several moving violations. The defendant fled on foot carrying a soda bottle with liquid, and smelled of marijuana. Laboratory analysis of the liquid confirmed positive for codeine, while analysis of the urine detected codeine, promethazine, marijuana metabolite and alprazolam.

As this laboratory system continues to analyze for codeine and other related drugs, it is noted that a substantial abuse pattern in the greater Houston area during a relatively short period of time. In accordance with the toxicological cases presented, there is a tendency of abusing other drugs in addition to codeine. Knowing the behavioral and toxicological effects of liquid codeine, the community and law enforcement agencies must be educated about the prevalence of this unsuspecting cough syrup. The authors recommend that crime laboratories incorporate standardized drug screening to include liquid codeine for drug or alcohol related incidents.

Opiates, Liquid-Codeine, Harris County Medical Examiner Office

K25 The Toxicological Significance of Tramadol in Death Investigation and Impaired Driving Cases

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After attending this presentation the participant will be able to assess the toxicological significance of a positive finding of Tramadol (Ultram®). Tramadol is a synthetic opioid-receptor agonist that exerts additional effect by inhibiting the reuptake of norepinephrine and serotonin. It has been used in the United States since 1995. Clinically it is used to treat moderate to severe pain. It is reported to have minimal abuse potential, and only mild side effects. Tramadol taken alone has been reported to be a relatively safe drug. However, due to its mode of action it could be potentially lethal when taken in excess or taken together with drugs acting on the same neurochemical or metabolic pathway.

This work was conducted as part of an assessment of the increasing incidence of analgesic drugs in the death investigation casework in the State of Washington between 1995 and 2000. Drug concentrations were assessed relative to accepted therapeutic ranges, and compared to concentrations encountered in drivers arrested for apparent impaired driving. Patterns of drugs found in combination with tramadol were assessed to identify possible pharmacokinetic and pharmacological interactions.

To perform this assessment, we obtained toxicological data from all death investigation cases in which tramadol was present, and matched it with public health data listing the certified cause and manner of death (n=72). All cases were tested at the Washington State Toxicology Laboratory for alcohol and screened for general drugs of abuse. Confirmation and quantitation of tramadol and other drugs was performed by gas chromatography/mass spectrometry. Additionally, investigative and autopsy data were obtained from the three largest counties in the state, and were used to examine the circumstances of death, and pathological features associated with a subset (n=40) of these cases. The toxicological data from a series of DUI cases occurring over the same period (n=39) were also considered This was used as a control group (living subjects) to assess postmortem drug concentrations and drug combinations. Literature on clinical trials of tramadol was also reviewed to assess normal patterns of prescribing and blood concentrations.

We observed a significant upward trend in the number of deaths certified in Washington State, involving tramadol, increasing from 5 cases in 1995, to 24 cases in 2000. The concentrations observed in death investigation cases ranged from <0.05 to 22.2 mg/L (mean: 2.06 mg/L, standard deviation: 4.02, median: 0.68mg/L). Toxicological literature states the therapeutic range to be 0.28-0.50 mg/L. For comparison, concentrations in suspected impaired drivers ranged from <0.05 to 5.36 mg/L (mean: 0.45 mg/L, standard deviation 0.94, median:0.15 mg/L). The most frequently observed manner of death was accidental (44%, 84% of which were "drug caused deaths") followed by natural (21%) suicide (18%, 77% of which were "drug caused deaths") and undetermined (12%, 92% of which were "drug caused deaths").

Tramadol was invariably ingested in combination with other drugs. In our data set there were no cases in which the cause of death was attributed to tramadol alone. Almost seventy percent of the cases were classified by the Department of Public Health as death attributed to drug(s). In those cases, other drugs were present in every instance. Morphine was the drug most frequently taken in combination with

tramadol, closely followed by amitriptyline, its metabolite nortriptyline, nordiazepam, acetaminophen, trazodone, and carisoprodol. Over half of the decedents (66% of the "drug caused deaths" and 55% of the non-drug caused deaths) were taking an antidepressant in conjunction with tramadol. Similar patterns were observed in the drivers in whom antidepressants were present in 38% of cases. There were several cases in which death was attributed to the combination of tramadol with other drugs affecting the reuptake of serotonin. These included tricyclic antidepressants, and the selective serotonin reuptake inhibitors (SSRI's) fluoxetine, and sertraline. As tramadol itself inhibits serotonin reuptake, this raises the possibility of a serotonergic crisis (e.g. serotonin syndrome) contributing to the actual mechanism of death. Another concern is a metabolic interaction between tramadol and amitriptyline, which are both metabolized by the cytochrome P4502D6 enzyme. This combination may contribute to an elevation of tramadol concentrations even with therapeutic administration.

Our data show that tramadol does appear to be a fairly safe drug when taken alone, and that patients can survive concentrations in considerable excess of the accepted therapeutic concentration, albeit with significant apparent psychomotor effects on motor skills. Patterns of prescribing of tramadol still appear to include the co-administration of drugs that may have significant metabolic or pharmacological interaction, and these should be carefully considered when interpreting postmortem toxicological data.

Tramadol, Drug Interaction, Postmortem Toxicology

K26 Simultaneous Determination of the Nerve Gases GB (Sarin) and VX and the Vesicant HD (Sulfur Mustard)

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The goal of this presentation is to present methodology for rapidly detecting exposure or contamination from the chemical warfare or potential terrorist agents, GB (sarin), VX, and HD (sulfur mustard).

The U.S. as a signatory to the Chemical Weapons Convention plans upon destroying the domestic stockpile of chemical warfare agents stored at six different sites by 2007. Some of these storage sites, such as the Pine Bluff (Arkansas) Arsenal, have substantial populations living near the demilitarization facility. In the unlikely event that an accidental release occurs, monitoring of persons potentially exposed and environmental contamination will be necessary for assessing effects on public health. The nerve gases GB (sarin) and VX are two of the chemical warfare agents currently scheduled for destruction. These toxic gases are relatively easy to synthesize and have previously been used for terrorist activities in Japan. Additionally, it is known that several rouge nations supporting terrorist activities also possess these nerve agents. The vesicant (blistering agent) HD in addition to being in U.S. stockpiles slated for destruction is also known to be in the possession of some rouge states. Therefore, having an assay for detection of these chemical agents becomes important in any forensic investigation following an incident.

GB and VX hydrolyze in the environment and are metabolized in humans by essentially identical pathways. These organophosphates form a common end product, methylphosphonic acid (MPA) which if identified would indicate that either of these agents was utilized. More specific identification was ascribed by determination of the immediate precursors to MPA, either isopropylmethylphosphonic acid (IMPA) or ethylmethylphosphonic acid (EMPA) derived from GB and VX, respectively. HD also undergoes environmental hydrolysis and human metabolism in identical manners and forms thiodiglycol (TDG) and thiodiglycol sulfoxide (TDGS). Therefore, an analysis method that can detect MPA, IMPA, EMPA, TDG, and TDGS can be utilized for multiple matrices by modifying the pre-analytical work-up.

A GC-MS method was developed that simultaneously detects MPA, IMPA, EMPA, TDG, and TDGS as their respective silylated derivatives in a 10-minute analysis. For urine analysis, 100 µL of a 1,000 ng/mL aqueous solution of d_7-IMPA and d_8-TDG was added as internal standards to 3 mL of urine. Calibrators containing 3.1, 6.3, 12.5, 25, 50, and 100 ng/mL of MPA, IMPA, EMPA, TDG, and TDGS in laboratory workers' urine were used to determine replicate urine specimens to which 0, 10, and 80 ng/mL of these hydrolysis compounds were added. Following addition of the internal standards, 1 mL of 5% HCl was added followed by extraction with 3 mL 9:1 $CHCl_3$:Isopropyl alcohol and centrifugation to separate the organic layer that was evaporated to dryness under nitrogen at 50°C. To the resultant residue was added 30 µL BSTFA and 70 µL ethyl acetate followed by heating at 75°C for 15 min. GC-MS conditions were as follows: injection volume 1 µL; injector port 180°C; interface 280°C; column, HP-1 (12m x 0.2 mm i.d.); oven program 50°C for 4 min, 40°C/min, 280°C for 0.25 min; helium flow 0.5 mL/min; SIM mode with 50 ms dwell; and EM 400 volts above daily tune. Retention times and ions (where *q* is the quantitative ion) were: EMPA, 6.02 min, *m/z* 153 (*q*), 154, 137; d_7-IMPA 6.15 min, *m/z* 154 (*q*), 171, 155; IMPA, 6.17 min, *m/z* 153 (*q*), 195, 169; MPA 6.39 min, *m/z* 225 (*q*), 226, 227; d_8-TDG 7.81 min, *m/z* 119 (*q*), 183, 168; TDG 7.83 min, *m/z* 116 (*q*), 176, 130; TDGS 8.64 min, *m/z* 166 (*q*), 117, 267.

The LOQ of the developed method was 3.1 ng/mL for all the analytes of interest and the LOD was 1.5 ng/mL. Because exposure of humans to the nerve gases and vesicant constitute unethical experimental paradigms, validation of the method will require the determination of a baseline levels of the compounds in a substantial number of non-exposed humans. TDG is known to occur at low levels in human urine as a by-product of dietary habits. Once a background-level for all the analytes is determined, a level of 2SD above the mean could be used for indicating exposure.

Nerve Gases, Vesicant, Urine Analysis

K27 A Fatality Due to Lorazepam and Morphine Intoxication During Long Term Therapy

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The objective of this presentation is to report the concentration and distribution of both lorazepam and morphine in various specimens collected from a single fatality after documented chronic high dose treatment with both drugs for 17 days.

Content: A 48-year-old male was brought to the emergency room complaining of chest pain/discomfort due to an alleged assault he had sustained. Hospital records failed to document evidence of the assault, a CT scan of the chest showed a "spot" on the lower lobe of the left lung that was described as a small pulmonary contusion. He was subsequently admitted to hospital, intubated and dosed IV with morphine, lorazepam and propofol to sedation with plans to biopsy the lung for possible pathogens. The patient had a history of active HIV with a low CD_4 count. The hospital also documented pneumonia. The patient was on HIV medications and arrived at the hospital alert and talking. Multiple biopsies, tissue cultures and other studies with special stains failed to yield the pathogens usually seen in AIDS such as pneumocystis carinii and cytomegalovirus. Antibiotics were administered for the pneumonia and the patient remained on a ventilator for the entire course of his hos-

pital stay. The original doses of morphine and lorazepam began at 2 mg per hour for both drugs and increased over the term of his hospital stay (37days) until they reached 20 mg IV per hour where they remained for his last 17 days of life. The orders regarding the increase of both drugs were to "titrate to sedation" or "to any signs of discomfort." The family had agreed to sign a do not resuscitate order, and on the last hospital day the patient was extubated and died approximately 5 hours later. Prior to extubation the dose of both lorazepam and morphine was not discontinued nor was it decreased. At autopsy the deceased was cachectic at 126 pounds and 6 feet in height. Autopsy findings were unremarkable as no cause of death could be determined, and no evidence of recent or remote blunt trauma was observed. Specimens obtained at autopsy were evaluated for the presence of drugs and alcohol. Positive toxicology results are listed in

Table 1.

	Femoral Blood	Bile	Kidney	Spleen	Vitreous Humor
Lorazepam	5.8 mcg/mL	44 mcg/mL	39 mcg/g	12 mcg/g	1.0 mcg/mL
Morphine	1.6 mcg/mL	6.6 mcg/mL	1.3 mcg/g	0.5 mcg/mL	
Metoclopramide	QNS	Positive	TNP	TNP	TNP
Trimethoprim	QNS	Positive	TNP	TNP	TNP

QNS – Specimen quantity not sufficient for analysis
TNP – Test not performed

The toxicology findings were remarkable for the presence of free lorazepam and free morphine at markedly elevated concentrations. The cause of death in this case was acute lorazepam and morphine intoxication and the manner of death is undetermined. Free lorazepam was quantitated using solid phase extraction with subsequent derivatization with MTBSTFA with 1% t-BDMCS and EI-GC/MS analysis in SIM mode. The LOD for lorazepam is 12.5 ng/mL with a linear range up to 400 ng/mL and a %CV of less than 10. The free morphine was quantitated by liquid:liquid extraction with subsequent derivatization with MSTFA and EI-GC/MS analysis in SIM mode. The LOD for morphine is 12 ng/mL with a linear range up to 1000 ng/mL. This case raises serious ethical concerns about the practice of comfort care for those patients who are not immediately terminal.

Lorazepam, Morphine, Fatality

K28 Dihydrocodeine-Related Deaths: A Ten-Year Review

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The goal of this presentation is to present a retrospective analysis of dihydrocodeine (DHC) related deaths in the Yorkshire and Humberside regions of the U.K. over the ten-year period 1991-2000, to determine the fatal concentration of the drug in such deaths and to compare the results with experience in the published literature.

DHC is a semi-synthetic opioid drug similar in structure and biotransformation pathway to codeine. It is licensed for use in the U.K. as an analgesic and antitussive drug but over recent years it has found increasing popularity as replacement therapy for heroin and morphine addicted patients, and amongst illicit drug users. Its increasing use in the treatment of opioid drug addiction is thought to relate to its short half-life (4 hrs), less severe withdrawal symptoms and relative lack of potency in comparison with methadone. In common with codeine, some of the DHC metabolites are pharmacologically active, and may contribute to the development of tolerance. Relatively few studies have been published with respect to the pharmacology and toxicology of DHC, although recent literature from the UK and continental Europe points to a general increase in drug deaths in which DHC has been a significant contributory factor. There has been a wide range in the reported DHC levels in these cases, thought mostly to be due to the synergistic effect of other drugs, varying degrees of tolerance and the genetic control of some metabolic steps.

The postmortem records of all cases in which DHC was detected on toxicological analysis were reviewed for the period 1991-2000. A total of 250 such deaths were identified and the vast majority of these cases were suspected overdoses where a full screen was routinely performed. DHC was detected in the blood in trace amounts in 12%, within quoted therapeutic levels in 47%, higher than therapeutic levels in 12% and potentially fatal levels in 29% (n = 72). In those fatalities where DHC was considered the sole or major contributor to the cause of death, the mean age was 42 years (range 16- 73 years) and there was a male predominance. The range of DHC concentrations in these fatalities was 0.4- 68.7 mg/L (mean 9.7), and in over half of the cases the concentration was less than 10 mg/L. DHC was detected in combination with alcohol and/or other drugs in all such cases and of the 70 cases where analysis of blood alcohol was performed, the level ranged from 6- 481 mg/100ml. Of the other drugs detected, the most commonly encountered were, not surprisingly opioids (63 cases). The effect of other drugs with central nervous system-depressant qualities, and the development or loss of tolerance has made a quantitative assessment of a 'fatal' DHC level problematic, and similar problems beset any analysis of opioid related deaths, nevertheless, the striking feature of this review was the marked increase in cases where DHC was detected over the study period, and, concomitantly, in the number of cases were death was attributable, at least in part, to DHC toxicity. A significant influence on these figures has no doubt been the increasing prevalence of the drug amongst illicit drug users and its increased prescription by individual physicians. The numbers of cases appear to have reached a plateau by the end of the decade, but nevertheless remain high. In fact, due to the 'targeted' nature of toxicological screening in the jurisdictions covered by this study, the true incidence of DHC-related deaths is most probably significantly under-reported, particularly with respect to road trauma, where blood alcohol is the only analysis performed in many cases.

In conclusion, this study shows that DHC has assumed increasing prominence in drug overdose deaths over the past decade. Possible over-prescription, poor supervision and increasing prevalence amongst illicit drug users are all causes for concern.

Dihydrocodeine, Opioids, Autopsy

K29 Fatal Ethylene Glycol Intoxication in the State of Maryland for the Last Seven Years

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The goals of this presentation are to present the audience with the most recent trends in the state of Maryland regarding ethylene glycol (antifreeze) intoxication, and to describe the distribution of calcium oxalate crystals in different tissues.

The state of Maryland OCME has investigated ten deaths caused by ethylene glycol (EG) intoxication in the past seven years. This incidence, in a state with a population of about 6 million people, is comparable to that of the whole country, where 40 to 60 deaths caused by EG intoxication are recorded yearly. Most cases involved intentional ingestion by adults with psychiatric illnesses, mainly depression and ethanol dependence.

EG is a relatively nonvolatile (odorless), slightly sweet tasting liquid utilized for its thermal properties, in antifreeze and coolant solutions. It has a half time of three hours and is metabolized by the liver to three major compounds: glycoaldehyde, glycolic acid and glyoxilic

acid. Oxalic and formic acids are formed in smaller amounts. Glycolic acid is the main compound responsible for the metabolic acidosis. Oxalic acid binds calcium and precipitates into calcium oxalate in tissues. As little as 100 ml EG are usually lethal in adult humans. EG has been responsible for fatal and non-fatal accidental poisoning of subjects following contamination of the water supply. An antidote for ethylene glycol poisoning, Fomepizole (Antizol, Orphan Medical Inc.) was approved by FDA in December 1997. It inhibits the formation of toxic metabolites.

Methods: The selected cases studied at the OCME were death was due to EG intoxication in a seven year period, and reviewed the clinical and demographic characteristics of the subjects, together with the scene investigation and autopsy findings, including toxicology and histology. Ethylene glycol may be determined in tissues by calorimetric method. The gas chromatographic method was used in the study. Both, the gas and liquid chromatography, such as High Performance Liquid Chromatography (HPLC) are more specific methods for quantitation of ethylene glycol and glycolic acid and confirmation of the diagnosis.

Results: There were eight men and 2 women, aged 13 to 73 years. Scene investigation suggested the possibility of EG ingestion, and this was confirmed by toxicologic analysis performed at the Medical Examiners Office or hospital (7 cases), and microscopic findings and scene investigation (3 cases). All cases indicated oral ingestion, and the manner of death was ruled as suicide in six cases. The manner of death was ruled as undetermined in four cases due to lack of a strong evidence for a suicidal attempt. Less than half of the suicide cases wrote a note of intent (comparable to the experience with other methods of suicide). Five subjects had clinical depression and four were ethanol abusers as well. One subject had schizophrenia. Autopsy showed nonspecific findings, with brain swelling present in some individuals. EG level in blood ranged from 0 mg/dl to 1700 mg/dl (mean = 266.91 mg/dl, median =43.9 mg/dl). Calcium oxalate crystals were visualized in the histologic sections of the kidney in all subjects. Similar crystals were seen in sections of the brain and rarely of lung and other tissues in some individuals.

EG Intoxication is an uncommon but recurring method of suicide. EG is a toxic, inexpensive and easy to obtain material. In the experience, EG is rarely, if at all, involved in domestic accidental deaths in either adults or children. Although the presence of oxalate crystals in the kidney has been reported in the literature, the presence in the brain and other tissues has not been so widely recognized.

Ethylene Glycol, Calcium Oxalate, Intoxication

K30 Gabapentin, A Novel Adjunctive Agent: Case Review of Twenty-Two Postmortem Toxicology Investigations

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This presentation will discuss the clinical uses and role of gabapentin in postmortem forensic toxicology.

Gabapentin (Neurontin) is indicated as an adjunctive antiepileptic drug (AED). It has been in clinical use since 1993 for the treatment of complex partial seizures. Although conventional AEDs such as carbamazepine, phenytoin and valproic acid continue to be the mainstay of clinical management, new generation therapeutics like gabapentin have emerged as useful adjuncts with low interaction potentials and improved tolerability. Gabapentin is not metabolized and does not bind to plasma proteins. Elimination of unmetabolized drug occurs via the renal route which likely accounts for the lack of hepatic drug interactions that are common to some of the other new anticonvulsant drugs (felbamate, lamotrigine and topiramate).

Gabapentin is a novel antiepileptic agent that binds to voltage-dependent calcium channels. However, the exact mechanism of action is somewhat elusive. Gabapentin was originally indicated for the treatment of partial epilepsy, but recent investigations into the adjunctive potential of gabapentin have resulted in more widespread use for other disorders including pain management, psychiatric illness and bipolar disorder. A review of the literature indicates potential uses for movement disorders, migraine prophylaxis and cocaine dependence.

Since the 1990s, gabapentin emerged as an alternative chronic pain treatment. It is used for the management of diverse symptoms associated with neuropathic pain in combination with other therapeutic agents. In particular, gabapentin is reported to increase the analgesic effect of morphine, indicating a pharmacodynamic interaction. Morphine pharmacokinetics are apparently unaffected, but gabapentin concentrations are reported to increase when used in combination with the opiate, indicating a pharmacokinetic effect.

The pharmacodynamics of gabapentin are not well understood, but studies have shown that in combination with morphine, it blocks dopamine release from the nucleus accumbens, and as a result, may have some clinical utility for the treatment of opioid dependence. There have been relatively few reports of drug interactions or toxicities associated with gabapentin. However, the correlation between blood concentration and clinical efficacy is unclear. This, in combination with the increased use of the drug as an adjunct for the treatment of nonepileptic disorders warrants further pharmacological investigation.

A total of twenty-two medical examiner cases involving gabapentin were reviewed. Postmortem blood concentrations ranged from 3 – 130 mg/L and all but one case involved multiple drug use. Case histories and toxicology results were consistent with adjunctive therapy for seizure and pain management. Common combinations included concomitant use of gabapentin with opiates (n=14), selective and nonselective serotonin reuptake inhibitors (n=13) and benzodiazepines (n=11). The majority of cases were attributed to accidental death. Case histories and toxicological findings are reviewed.

Gabapentin, Postmortem Toxicology, Blood

K31 A Case of Fatal Difluoroethane Intoxication

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Attending this presentation will enable the participant to learn about the analysis of difluoroethane and its tissue distribution in a post-mortem case.

1,1-Difluoroethane (DFE, halocarbon 152A, Freon 152) is a colorless, odorless gas that is used as a cooling agent, in aerosols and in the manufacture of other chemicals. Inhalation of DFE can produce coughing, shortness of breath, pulmonary edema, headache, dizziness and loss of consciousness. The intentional abuse of DFE for its intoxicating effect has been reported.

A case was received at the State of Delaware Office of the Chief Medical Examiner involving the intentional abuse of DFE. The decedent was a 34-year-old white male found in a prone position in a concrete drainage culvert with a head injury and his face partially submerged in water. An empty can of dust-off was found under the decedent's leg. Further investigation of the scene revealed a Walkman, cigarettes and 13 empty cans of cleaning duster spray. At autopsy, mul-

tiple specimens were collected including heart blood, brain, liver, bile, urine, gastric contents and vitreous humor. Specimens were stored in sealed polypropylene specimen cups at 4°C until analysis. In addition, a 10-mL aliquot of heart blood was sealed in a 20-mL headspace vial and frozen until analysis for DFE. Routine toxicological screening of heart blood and urine for alcohol and drugs of abuse yielded negative results.

It was learned from the decedent's father that the decedent had a long history of drug and alcohol abuse and for about the past year he had been abusing inhalants. Two different brands of cleaning duster spray were found at the scene, one containing DFE and the other containing 1,1,1,2-tetrafluoroethane (TFE). Analysis of DFE and TFE standards and the decedent's heart blood by gas chromatography-mass spectrometry indicated only the presence of DFE in the decedent's heart blood. Multiple postmortem specimens were analyzed for DFE by dual column (Restek BAC1 & BAC2) headspace gas chromatography with flame ionization detection. Quantitation was performed with a 9-point calibration curve ranging from 0.8 mg/L up to 204 mg/L DFE using n-propanol as an internal standard. A stock DFE standard was prepared by weighing DFE into 5 mL of methanol in a sealed 20 mL headspace vial. After determination of the methanol-air partition coefficient for the DFE in the vial, the concentration of the stock standard was calculated. Calibrators were prepared by spiking blank blood with the stock standard. Quality controls at concentrations of approximately 4, 40 and 400 mg/L were included with each batch. Blood, vitreous, urine, bile and gastric contents (0.1 mL) were diluted with internal standard solution (1.0 mL) and sealed in a 20-mL headspace vial. Tissue specimens were homogenized with internal standard solution and added to a 20-mL headspace vial. The concentration of DFE in the various specimens analyzed are summarized in the table below:

Specimen	DFE (mg/L or mg/kg)
Heart Blood	413
Vitreous Humor	342
Brain	133
Liver	91
Bile	256
Urine	104
Gastric Contents	272

Interpretation of the quantitative results for DFE is made difficult by the lack of published clinical or postmortem data for DFE. In a single published case report, blood concentrations of 78 mg/L and 35 mg/L DFE were reported in a driver and a passenger involved in a fatal automobile accident [(1)]. The presence of high concentrations of DFE in the decedent's heart blood and tissues and the evidence collected at the scene in this case suggest that the decedent was inhaling DFE from the cleaning duster spray close to the time of his death. It is suspected that he lost consciousness due to his intoxication with DFE, causing him to fall and strike his head, ultimately landing with his face submerged in water. The cause of his death was ruled inhalation of difluoroethane complicated by blunt force head injury and drowning. The manner was ruled undetermined due to the unknown cause of the head injury.

1. Broussard LA, Brustowicz T, Pittman T, Atkins K, Presley L. Two traffic fatalities related to the use of difluoroethane. *J Forensic Sci* 1997;42(6):1186-1187.

Difluoroethane, Analytical Toxicology, Postmortem

K32 Coexistence and Concentration of Ethanol and Diazepam in Postmortem Blood Specimens

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The objective of this presentation is to present toxicological results from the analysis of postmortem blood specimens and to discuss the potential for toxic interactions between two of the most commonly used psychoactive drugs, namely ethanol and diazepam.

Serious drug interactions involving alcohol are not uncommon and these have accounted for many fatal poisonings. The combined effect of alcohol and barbiturates was notorious and these two central nervous system (CNS) depressants caused countless deaths by accidental overdose and by suicide. Another dangerous drug-alcohol combination arises with the pain-killer propoxyphene. Even drugs from the benzodiazepine group, such as diazepam and flunitrazepam, despite their reputation of low toxicity in overdose have been implicated in many deaths, especially when used together with a large dose of ethanol.

Notwithstanding the difficulties in interpreting the results of postmortem drug concentrations, owing to the many factors that must be considered, the authors evaluated the coexistence and concentrations of ethanol and diazepam in postmortem femoral venous blood samples. The toxicological results are discussed in relation to the pharmacology of ethanol and diazepam and the risk for toxic response when these CNS agents are taken together.

We located 234 autopsy cases when ethanol and diazepam with or without its primary metabolite nordiazepam were the only drugs present. All blood specimens were taken from a femoral vein and the analytical toxicology was done at one central laboratory, the National Laboratory of Forensic Toxicology (Linköping, Sweden). The concentration of ethanol in blood was determined by headspace gas chromatography on two different stationary phases and the mean concentration was reported with a limit of quantitation (LOQ) of 0.01 g% in routine casework. Diazepam and its metabolite nordiazepam were determined simultaneously in whole blood by capillary column gas chromatography after solvent extraction without derivatization. The GC instrument was fitted with a N-P detector and the method LOQ was 0.03 mg/L for both diazepam and nordiazepam.

The distributions of diazepam and nordiazepam concentrations in blood were markedly skewed to the right and in the vast majority of cases diazepam concentrations were within the therapeutic range of 0.07-0.42 mg/L for whole blood, according to the TIAFT listing. In this material, only 10 cases (2.5%) contained a diazepam concentration above 0.8 mg/L, which is considered the lower point of the toxic range. The highest concentration of diazepam was 2.6 mg/L. The concentrations of diazepam and its primary metabolite nordiazepam were highly correlated ($r = 0.73$, $p<0.001$). By contrast, the concentrations of ethanol and diazepam were not at all correlated ($r = -0.15$, $p >0.05$). The mean blood-ethanol concentration was 0.23 g% (median 0.25 g%), which confirms a high proportion of heavy drinkers in this forensic material. Indeed, 90 individuals (38%) had a BAC over 0.3 g%, which is approaching a dangerously high concentration even without the coexistence of another CNS depressant drug. Cases with blood-diazepam >0.8 mg/L were investigated in detail by checking the cause of death according to the pathologists report. Several instances of traumatic deaths were observed so the drug-alcohol combination cannot be considered as the cause but night have contributed to the death. There were other instances of nothing remarkable at autopsy except the presence of the two depressant drugs.

Many studies have demonstrated that small doses of ethanol and diazepam impair psychomotor skills more so than either drug alone. Ethanol and diazepam both cause sedation and their pharmacodynamic

interaction involves activation of the inhibitory $GABA_A$ receptor, opening of the chloride channel to elicit a tranquilizing effect on the individual. There is no strong evidence for pharmacokinetic interaction between ethanol and diazepam. Autopsy blood-specimens submitted for analysis are always hemolyzed and often contain clots. For drugs like diazepam, which are predominantly bound (>96%) to plasma proteins, the concentration in serum or plasma will be appreciably higher than in whole blood or erythrocytes. Postmortem toxicology results should not be compared directly with clinical pharmacology reports based on the analysis of plasma or serum. The plasma/whole-blood distribution ratio for diazepam is 1.8:1 (Clarke 1982). The blood-ethanol concentration required to cause death is generally considered to be 0.4-0.5 g% but this figure can vary widely depending on different circumstances such as age of the individual, development of chronic tolerance, inhalation of vomit, positional asphyxia, hypothermia and not least the combined use of other psychoactive drugs. When trauma can be excluded and no other complicating factors exist it seems reasonable to accept a high blood-ethanol concentration and a blood-diazepam concentration within the toxic range as the cause of death. The sedative effect of these two CNS depressant drugs is additive.

Ethanol, Diazepam, Interaction

K33 Postmortem Fentanyl Levels Following Chronic Administration With an Infusion Pump

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The goals of this presentation are to report the levels of fentanyl found in postmortem tissue and fluid samples from a patient chronically administered fentanyl, intravenously (IV), via a patient controlled analgesia (PCA) infusion pump.

Fentanyl is a synthetic narcotic analgesic routinely used as an adjunct in anesthesia or for the management of chronic pain in the form of a transdermal patch (Duragesic®). More recently, clinical studies have shown that fentanyl can be used as an alternative medication in continuous infusion to patients who require high doses or become refractory to traditional opioid treatments. Fentanyl is 50-100 times more potent than morphine and has a short duration of action. Fentanyl is not to be used for acute pain and is given in therapeutic doses of 25-100 micrograms/hr for chronic pain.

A case study is presented to document the postmortem levels of fentanyl found in an individual who had a history of chronic pain. The deceased, a 62 year old female Caucasian, was found dead in bed with an IV line to her subclavian vessel attached to a PCA from which she was being administered fentanyl.

Methods and Results: Fentanyl was extracted from the samples by solid phase extraction with an elution solvent of isopropanol/dichloromethane/ammonium hydroxide (18:80:2). The extracts were analyzed by electron ionization, gas chromatography/mass spectroscopy, operating in the selected ion monitoring mode, utilizing deuterated fentanyl as the internal standard. The following ions were monitored, *245*, 146, 189, *250*, 151, 194, with a calibration curve ranging from 5-100 ng/mL.

The results of the toxicological analyses performed for fentanyl are shown in the table below. The blood was also found to contain therapeutic levels of carisoprodol, meprobamate, fluoxetine, norfluoxetine, nordiazepam, and acetaminophen. In addition, hydrocodone was present at a level of 0.34 mg/L.

Source of Sample	Aorta	Vena Cava	Liver	Vitreous Humor	IV Bag
Fentanyl Conc.	100 ng/mL	95 ng/mL	64 ng/g	5 ng/mL	42,000 ng/mL

The patient had been prescribed fentanyl, administered by continuous infusion, intermittently for two years to treat chronic back pain and pain caused by pancreatitis. The dosing regimen usually began at 60 micrograms/hr and tapered off to 40 micrograms/hr over a few weeks. At the time of death, a dose of 40 micrograms/hr was being administered. The PCA prescription also allowed for a bolus dose to be delivered at a rate of 5 micrograms/6 min, resulting in a maximum dose of 90 micrograms/hr. According to the PCA program, the dose was last adjusted three weeks prior to death. Other drugs detected during toxicological analyses were present at concentrations consistent with the prescribed dosing of the patient.

There is limited information published regarding fentanyl delivered to chronic-pain patients via continuous infusion, IV. A review of the literature shows that fatal overdoses have been reported in the range of 3-139 ng/mL of fentanyl in heart blood, with cases as high as 800 ng/mL. Corresponding liver values are generally 2-7 times higher than the heart blood. However, these results pertain to the Duragesic® transdermal patch or acute dosing of the drug by self-administered intravenous injection or oral ingestion.

The toxicological findings on the blood were high by therapeutic standards although the cause of death was not attributed to an overdose of fentanyl. The patient was not a naïve user of fentanyl, or other opioids, and the corresponding liver and vitreous fentanyl levels were not proportionally as high as the blood. The patient had previously tolerated the current dose of fentanyl, and higher. The patient also had a history of chronic respiratory illness and lupus. It is recommended by the authors that a complete toxicological analysis be performed on all available specimens, and that a comprehensive history be gathered before drawing a conclusion as to the cause of death, especially on the basis of one blood value alone.

To the authors' knowledge, this is the first reported case of postmortem fentanyl concentrations after administration via a continuous infusion pump.

Fentanyl, Infusion Pump, Overdose

K34 Alcohol Exposure in Neonates

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Attendees will learn about the analysis of biomarkers in meconium for detection of fetal alcohol exposure.

Fetal alcohol syndrome (FAS) is a devastating disorder in the newborn, resulting from heavy maternal alcohol consumption during pregnancy and is the leading cause of non-hereditary mental retardation in the neonate. Estimates of the prevalence of FAS range from 0.5 to 3 per 1,000 live births in most populations. Children with significant prenatal alcohol exposure do not always exhibit the characteristic facial abnormalities associated with FAS, but still have mental impairments just as serious. Alcohol related neurodevelopmental disorder (ARND) and alcohol-related birth defects (ARBD) describe these conditions which are estimated to affect 3-4 times as many babies as FAS. The diagnoses of ARND and ARBD require confirmation of the mother's alcohol use during pregnancy in addition to psychological or neurological assessment of the child. Self-reported maternal history of alcohol use can be helpful in diagnosis, but a laboratory test may provide the physician with critical information, especially when an accurate maternal self-report is missing. Fetal exposure to alcohol can also cause CNS dysfunction, post-natal growth problems, cardiac defects and

attention deficit disorders in the neonate. To date, diagnosis of fetal alcohol effect depends largely on maternal interview, although clinical tests are becoming more widely used.

Fatty acid ethyl esters (FAEE) are formed in the body, by esterification of ethanol with free fatty acids and trans-esterification of glycerides; and have been detected in the meconium of newborns.

This paper estimates the prevalence of fetal alcohol exposure in two populations by detecting fatty acid ethyl esters in meconium. The prevalence of FAEE's in the meconium from two separate groups of neonates using solid-phase extraction and analysis by gas chromatography-mass spectrometry in chemical ionization mode is presented.

Methods: *Extraction:* Fatty acid ethyl esters are sensitive to heat and light, and therefore, it is recommended that meconium specimens be immediately stored in amber or opaque containers upon collection, be shipped on ice and be stored frozen (-20°C). Meconium (0.5 - 1g) was allowed to thaw, and was homogenized in organic solvent. The extract was centrifuged and the supernatant passed through a solid-phase extraction cartridge. The fatty acid ethyl esters were eluted from the column, and evaporated to dryness under nitrogen at 37°C. The dried extract was reconstituted in hexane and analyzed using full scan chemical ionization GC/MS, with acetone as the reagent gas.

Analysis*:* A Varian Star 3400 bench top GC coupled to a Saturn II ion trap mass spectrometer was operated in the full scan positive chemical ionization mode. The GC column was a bonded phase fused silica (0.25 mm ID; 0.25 mm film thickness; 30 m length). The injector was operated at 250°C in splitless mode and the injection volume was 3 mL. The oven was programmed to 310°C and the reagent gas was acetone. Chemical ionization (CI) was chosen for this analysis, because electron impact ionization of these compounds yields identical fragments for the various FAEE's. In CI mode, a diagnostic ion for each compound is obtained.

Results: In the first study, seventy-three (16.7%) of the meconium specimens tested (n = 436) were considered to be positive for FAEE's. When broken down into quartiles, the mean values of total FAEE's measured were 1059 ng/g; 3133 ng/g; 6628 ng/g and 62115 ng/g. In the second study, thirty-five (11.9%) of the specimens (n = 292) were considered positive. When broken into quartiles, the mean values were 1139 ng/g; 3067 ng/g; 7674 ng/g and 50,143 ng/g. The overall FAEE profiles of the two study sets were remarkably similar.

Summary: When the total FAEE concentration is greater than 10,000 ng/g, in an adequate meconium specimen, it is likely that the newborn has been exposed to significant amounts of alcohol during pregnancy.

Meconium, Fatty Acid Ethyl Esters, Fetal Exposure to Alcohol

K35 A Case of Repeated Tramadol Poisoning in an Infant

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During this presentation, the participants will learn about the metabolism of tramadol and its incorporation into hair. The objective of this report is to show the potentials and pitfalls of interpreting results from segmental hair analysis and the use of toxicological analysis of several matrices to support an expert opinion.

In January 2001, a five-month female infant was admitted to the emergency room (ER) with lowered consciousness and convulsions. During early spring 2001, the infant had four additional admissions to ER with the same symptoms and also small pupils and respiratory depression. Besides toxicological screening at the hospital a neurological evaluation was also performed. The toxicological screening was negative and the neurological tests were normal. At this point, the lab was contacted and it was decided that in case of another ER visit, samples would be taken and sent to the National Board of Forensic Medicine for analysis. Nothing happened until November 2001 when the now 15-month-old girl was admitted to the ER with similar symptoms as before. Serum and urine samples were obtained and sent to the forensic laboratory in Linköping for analysis, and a police investigation was initiated. When the opioid tramadol together with its metabolites N-desmethyltramadol (N-dm-T), and O-desmethyltramadol (O-dm-T) were identified in the girls' serum and urine, poisoning was suspected and because of the earlier ER visits a hair sample was obtained to find out if tramadol had been administered more than once. The girl's hair had never been cut. A search for other samples taken during the spring 2001 was also initiated.

Experiment

Hair was segmented (10 mm each), washed and weighed in screw-capped glass tubes. One mL of 1 M potassium hydroxide was added and the hair sample was heated at 80° C for 10 minutes with occasional shaking. After cooling to room temperature the sample was extracted with 3 ml of a mixture of dichloromethane:isopropanol (80:20) containing 20% pentane. To serum, spinal fluid, and urine 0.1 ml of potassium hydroxide was added before the extraction.

After centrifugation for 5 minutes at 4200 *g* 2.7 ml of the organic phase was aspirated and transferred to a new 10-mL screw-capped glass tube and the sample was evaporated under a gentle stream of nitrogen at room temperature. The sample was then reconstituted in 100 µL of mobile phase, and transferred to a vial. Liquid chromatography-tandem mass spectrometry with an electrospray interface was used for analysis. The transitions monitored were 264.1/58.1 for tramadol, 250.1/44.0 for N-dm-T, and 250.1/58.1 for O-dm-T. Calibration was performed as duplicates at 5, 10, 15, 20, 50, and 75 (ng) by addition of the analytes to 20 mg drug-free hair (obtained from a laboratory employee) or 0.1 ml donor serum or drug free urine.

Results and discussion

Results from body fluids are shown in the table below and the results from segmental hair analysis are shown in the figure.

Date	Matrix	Tramadol (µg/mL)	O-dm-T (µg/mL)	N-dm-T (µg/mL)
01-01-14	spinal fluid	0.14	0.06	not detected
01-01-26	serum	0.56	0.14	0.07
01-11-19	serum	1.06	0.22	0.31
01-11-19	urine	present	present	present

The spinal fluid sample was taken during the first admission and the first serum sample was obtained during the second ER visit. Both were sent to the laboratory after the police investigation was initiated. Both samples had been stored in freezers at the hospital. The last serum and corresponding urine sample were taken during the latest ER visit and the samples were sent directly to the laboratory. All samples contained tramadol together with at least one metabolite but no other drugs (based on a neutral and a basic extraction followed by GC-NPD). Thus, tramadol might have been the cause of intoxication in all these three admissions to the ER.

During the investigation, one of the parents was suspected of having poisoned the infant on all six occasions. Before prosecuting for attempted murder, the prosecutor wanted to know if any other proof of tramadol administration could be obtained to include the three admissions in February-March when no samples were available. Hair samples from the girl were thus obtained in late February 2002, more than a year after the first ER visit.

The segmental analysis of hair showed the presence of tramadol in all segments, suggesting continuous administration of tramadol, though with changes in dose. The segments S3 and S4 represent October/November 2001 when the latest visit to ER occurred. The positive results from serum and urine taken at this time confirm tramadol

intake. Also, the positive serum and spinal fluid specimens confirm the positive hair segments S13/S12 (January/February 2001). Still, the positive segments S11-S5 may indicate yet other intoxications after the last ER visit March 1st –6th. On the other hand, the effect of dormant hair may produce positive results even though the administration of drug had stopped several months earlier. Hair that continues to grow after termination of drug intake will push the positive segments farther out and leave behind drug free hair. However, hair that stops growing at any time during intake will stay positive in the proximal segments, thus causing a "lag time" for the hair to be totally negative. This can be illustrated by examining the segment S2 representing time when the girl was in protective care and could not possibly have been given tramadol. In conclusion, the different samples and matrices together with the symptoms complement each other to strengthen the opinion that tramadol was the cause of the intoxication on all six occasions but the positive hair segments S11-S5 does not necessary indicate additional intake of tramadol during this period. Finally, the close cooperation between clinical and forensic toxicology units is of paramount importance for quick and accurate diagnosis of poisoning.

Tramadol, Hair, LC-MS-MS

K36 Pediatric Postmortem Toxicology: Involvement of Diphenhydramine in a Child Death

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After attending this presentation, the attendee will: 1) appreciate the importance of drug testing in all child deaths, (2) understand the factors which must be considered when evaluating the role of drugs in deaths of the young, and (3) possess information regarding drug concentrations in postmortem specimens from a pediatric case.

Diphenhydramine (DPH) is an antihistamine present in many medications available for the relief of allergic responses. It is also a component in combination medicines for congestion, colds and sinus headaches or as a component of itch stopping cream/gel or spray. It is available as a liquid, in chewables, tablets, caplets, capsules, and gelcaps. Recommended doses for formulations available for adults are typically 25-50 mg DPH HCl. These medications are contraindicated for children less than 12 years of age. Peak plasma concentrations in clinical adult specimens are typically <0.15 mg/L after oral administration.

Allergy medications for young children may be in liquid form and one teaspoon usually contains 12.5 mg diphenhydramine hydrochloride (HCl). The recommended dose for one formulation for a child 12-23 months with a weight of 18-23 lbs is 3/4 teaspoon or 9.3 mg DPH HCl every 4 hours. The indications for use of this drug are symptoms associated with hay fever and other respiratory allergies including sore/itchy throat, itchy/watery eyes, runny/stuffy nose, and sneezing. This case report describes the death of a young child while at the home of her childcare provider involving the administration of diphenhydramine.

A 17-month-old white female, weighing 26 lbs, was found dead in a playpen crib in an upstairs bedroom of the care provider's house. The crib contained numerous blankets and nylon carrying bags. According to the care provider the child was found unresponsive with a bed sheet tangled around her neck. CPR was initiated without success. The body was transported to the Office of the Cuyahoga County Coroner for autopsy. Autopsy findings included red petechiae over the left mastoid region and a linear transverse aggregate of red petechiae over the right anterolateral neck. Heart and femoral blood, cerebrospinal fluid, gastric contents, bile and vitreous humor were collected for toxicological analysis.

The heart blood was subjected to comprehensive toxicological testing which included volatiles by headspace gas chromatography; acetaminophen, salicylate and ethchlorvynol screening by colorimetry; acidic/neutral and basic drug screening by liquid-liquid extraction followed by GC-FID or GC-NPD with confirmation by GC/MS; benzodiazepine screening by GC-ECD; and modified opiate immunoassay screening. The only drug identified and quantitated was diphenhydramine. Due to the unusual circumstance of positive drug results in a young child and to understand issues of postmortem redistribution, the femoral blood, and gastric contents were also tested for diphenhydramine. DPH was detected at the following concentrations (mg/L): 0.49 heart blood, 0.27 femoral blood, and 0.36 mg in 20 mL gastric contents.

A search of the literature revealed little information on DPH pharmacokinetics in children and few cases of DPH detection in pediatric fatalities. Although the common side effect of DPH is drowsiness, it may also cause seizures in children. In light of the available literature and case circumstances, death due to a DPH overdose was discounted. Therefore, possible mechanisms to explain the child's death included entanglement in the bed sheet with inability to escape caused by the sedating effect of the drug or drug induced seizure with the ensuing entanglement. The cause of death was determined to be asphyxia due to entanglement by bed sheet around the neck, with other condition, recent ingestion of DPH. The death was ruled a homicide.

Pediatrics, Forensic Toxicology, Diphenhydramine

K37 Methamphetamine in Fetal and Infant Deaths in Washington State

Ann Marie Gordon, and Barry K. Logan, Washington State Toxicology Lab, Forensic Laboratory Services Bureau, Washington State Patrol, Seattle WA*

By attending this presentation the participant will learn about methamphetamine exposure in infants and children, and will receive guidance on how to interpret quantitative toxicological data.

Methamphetamine is a commonly abused drug in Washington State and positive methamphetamine findings in infant and fetal deaths have been increasing in recent years. Pediatric toxicology merits careful consideration, and caution in interpretation. Children cannot be treated as "small adults". Methamphetamine death in adults has been attributed to methamphetamine levels as low as 0.05 mg/L, however, this is usually in combination with other drugs or underlying disease. Pediatric methamphetamine poisonings are generally non-fatal, and amphetamine has been successfully administered to young children to treat hyperactivity disorders without adverse effects.

Several in utero deaths associated with maternal methamphetamine use have been reported but the significance of the methamphetamine concentration in these cases is often unclear, and can be controversial. In 1994, a California woman was convicted of child endangerment following the death of her two-month old infant son because she ingested methamphetamine and breast-fed her infant.

The authors reviewed fourteen cases of fetal and infant deaths with methamphetamine positive findings in autopsy blood, believed to be related to maternal methamphetamine use. Blood samples from the child or fetus was subjected to comprehensive toxicological screening including immunoassay and GC/GCMS analysis of both basic and weakly acidic fractions. Methamphetamine was detected in the basic fraction, following extraction with butyl chloride. The LOD for both methamphetamine and amphetamine was 0.02 mg/L, and limits of linearity were 0.02 - 10 mg/L for methamphetamine, and 0.02 - 5.0 mg/L for amphetamine.

The age of the infant and fetal deaths ranged from 22 weeks gestation, to 5 months old. 64% of the cases were stillbirths. The mean blood methamphetamine concentration in these pediatric death investigation cases was 0.24 mg/L (median, 0.18mg/L; range 0.04 - 0.59 mg/L) and mean amphetamine concentration was 0.07 mg/L (median, 0.06mg/L; range 0.02 - 0.16 mg/L).

A representative case was that of the death of an 8-week-old infant, found to have 0.04 mg/L methamphetamine, and < 0.01 mg/L amphetamine. The circumstances of this death were consistent with SIDS, and the family had a number of risk factors for SIDS (baby asleep on front, elevated temperature, history of child neglect, drug and alcohol use by the mother). While the methamphetamine was not clearly a cause of death in this case, it did however make SIDS (a diagnosis of exclusion) an inappropriate finding and the death was classified as undetermined in both cause and manner. No criminal charges were filed in this case. Charges were however filed in at least one other case.

As drug and chemical exposure of children in drug houses where methamphetamine is manufactured becomes an increasing concern, the authors also report the urine toxicological findings of two children exposed to methamphetamine. One 8-year-old was removed from a clandestine methamphetamine laboratory and had 0.04 mg/L methamphetamine and 0.02 mg/L amphetamine in his urine. The second child, a 1-year-old, was presented at an emergency room with signs of methamphetamine toxicity. His urine toxicology was positive for methamphetamine (15 mg/L) and amphetamine (0.9 mg/L).

Methamphetamine, Postmortem, Infants

K1 A Review of Postmortem Diltiazem Concentration

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The objective of this work was to determine an appropriate therapeutic range for diltiazem in postmortem blood specimens.

Diltiazem is a calcium antagonist drug used to treat angina, supra ventricular arrhythmias, and hypertension. The drug has a plasma elimination half-life of 3-6 hours. It is extensively metabolized by N-demethylation, O-deacetylation and O-demethylation, with less than 5% of the administered dose appearing in the urine as unchanged drug. The volume of distribution is 3-13 mg/kg. Steady-state plasma concentrations following therapeutic use are in the range of 0.1-0.3 mg/L.

Diltiazem is routinely identified in this laboratory by gas chromatography-nitrogen-phosphorus detection following an alkaline extraction of the biological specimen. No derivatization is necessary. The drug elutes late on a DB-5 column, following most antidepressants, antihistamines, benzodiazepines, and narcotic analgesics.

Since the drug is alkaline extractable and has a high volume of distribution, there was some concern about postmortem redistribution of the drug. As a result, cases investigated by the Office of the Chief Medical Examiner, State of Maryland, over a three-year period were reviewed to ascertain whether postmortem heart blood diltiazem concentrations may be interpreted on the basis of antemortem pharmacokinetic studies or whether a different "postmortem" therapeutic range is more appropriate. An additional aspect of the study was to determine whether there is significant site dependence for postmortem blood diltiazem concentrations.

Over the three-year period, 78 cases containing diltiazem were identified. Seven of the deaths were caused by diltiazem intoxication or multiple drug intoxication where diltiazem was considered a significant factor. In the diltiazem-only drug intoxication cases (n=5), the heart blood concentrations ranged from 2.1 to 42 mg/L. These blood concentrations were consistent with blood concentrations found in other diltiazem intoxication cases and were an order of magnitude higher than the antemortem therapeutic range.

To examine the postmortem therapeutic range, "therapeutic use" cases were identified. Cases were considered "therapeutic use" if the death was caused by an acute, non-heart event such as multiple injuries, gunshot wounds, narcotic intoxication, pulmonary emboli, or other acute natural deaths. Chronic disease states such as atherosclerotic or hypertensive cardiovascular disease or cirrhosis were not included. Forty-two deaths were classified as "therapeutic use" deaths. In these cases, the mean heart blood diltiazem concentration was 0.5 mg/L and the median blood concentration was 0.3 mg/L. The heart blood diltiazem concentrations in these cases ranged from 0.03 to 1.9 mg/L. The mean and median heart blood diltiazem concentrations in these cases appear consistent with the antemortem therapeutic range.

Both heart and peripheral blood were measured for diltiazem in 16 cases. The average heart blood to peripheral blood diltiazem concentration ratio was 0.90 and the median ratio was 0.92. The ratios ranged from 0.33 to 1.50. Ten of the 16 cases had a ratio between 0.7 and 1.3. Of the remaining 6 cases, the heart blood diltiazem concentration exceeded the peripheral concentration in only two cases.

From this study, it was concluded that the postmortem therapeutic range for diltiazem was similar to the antemortem therapeutic range. Furthermore, heart blood was a reliable specimen in the interpretation of postmortem diltiazem concentrations.

Diltiazem, Postmortem, Therapeutic

K2 Analysis of GHB and Its Analogs by Capillary Electrochromatography

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The objective of this analysis was to improve the methods of detection for gamma-hydroxybutyric acid (GHB) and its analogs using capillary electrochromatography with monolithic polymer columns.

Drug facilitated sexual assault has become more prevalent in today's society. GHB and its drug analogs are among the sedative hypnotic drugs used by assailants. The testing of these drugs by conventional methods is often tedious and imprecise due to the frequent time lapse between the attack and when the victim decides to report the crime. This judgment can be compounded by the amnesiac properties of GHB. Current methods are cumbersome and contain complex derivatization steps. The primary concern with GHB is the new popularity of its chemically related drug analogs. Gamma-butyrolactone (GBL) and 1,4-butanediol are more loosely controlled and more difficult to detect by conventional methods than GHB. By the time samples are taken concentrations may fall below detection limits. Capillary electrophoresis (CE) methods are currently being implemented as forensic screening techniques because of their advantages over GC-NPD. CE instruments require a small sample volume and use pre-concentration methods that make it ideal for trace levels of substances without the need for derivatization. Since capillary electrochromatography (CEC) columns can be used in these instruments, the development of these new CEC methods will provide an exceptional new application of CE and CE/MS systems.

CEC is able to use the charged electro-osmotic flow of CE, while incorporating HPLC packing material into the capillary. This gives a separation that involves a combination of electrophoretic mobility and partitioning. To keep the silica gel uniformly packed in the columns, frits are used, but can often lead to severe band broadening and reproducibility problems. An alternative to particulate HPLC packings is a rigid polymer structure that adheres to the capillary wall. These monolithic columns are relatively inexpensive, just as efficient as packed capillaries, and do not require the use of frits. The column procedure for this separation was modeled after the research of Dr. Frantisek Svec and colleagues from the University of California Berkeley, CA. The advantage of these columns is the simplicity of the three-step column preparation: surface modification, polymerization, and washing.

The surface modification washes the capillary and changes the surface charges to secure the monolithic attachment to the capillary wall. The polymerization mixture includes a monomer combination of butyl methacrylate (BMA), ethylene dimethacrylate (EDMA), and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS). The EDMA serves as the cross-linker for the methacrylate once the reaction is initiated by the presence of azodiisobutyronitrile (AIBN). The combination of these chemicals will produce a continuous cast inside the capillary with a rigid backbone of monomers in the presence of a fraction of pores. These include 1-propanol, 1,4-butanediol, and water to determine the permeability of the column and mass transfer effects with respect to band broadening. After the 12-24 hour incubation under UV light or in a 60-degree oven, washing is relatively simple. The porogens can be rinsed free of the capillary by either syringe pump pressure or introduction to the CE instrument.

The ultimate goal of this project is to achieve a complete separation of GHB, gamma-Butrylactone (GBL), 1,4-butanediol, and gamma-Aminobutyric acid (GABA). Many of the current laboratory methods involve a conversion of GHB to GBL. The CEC method can not only

distinguish all of the analogs, but also maintain a steady pH to prevent the interconversion of GHB to the lactone. The additional separating power of CEC permits the analyst to distinguish between the neutral compounds that elute simultaneously with traditional CE. CEC results with an 80% acetonitrile 20% phosphate buffer mobile phases will be compared to a CE method consisting of sodium phosphate buffer with UV detection. Different urine extraction techniques will be examined in order to accomplish the best quantitation of the drugs.

Capillary Electrochromatography, GHB, Date-Rape Drugs

K3 Simultaneous Determination of Underivatized Diazepam and Nordiazepam in Plasma Using Gas Chromatography/Mass Spectrometry

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A sensitive and simple gas chromatography/mass spectrometry method has been developed for the quantitative determination of diazepam and its major metabolite nordiazepam in plasma.

Diazepam (DZ) is one of the most frequently prescribed drugs as an antianxiety agent, muscle relaxant, and anticonvulsant that sometimes cause intoxication due to accidental overdose, misuse, or abuse. Furthermore DZ has been used for drug-facilitated sexual assault during the past few years. DZ is metabolized to nordiazepam (NDZ, desmethyldiazepam), oxazepam (OX), and temazepam (TM) which are also active, although OX and TM do not accumulate in blood or plasma to an appreciable extent. Screening or confirmation methods for DZ and NDZ in plasma are very important for clinical and toxicological studies and in forensic cases.

There are several literature methods available for the identification and quantification of DZ that are based on high performance liquid chromatography (HPLC) with various detection methods or gas chromatography/mass spectrometry (GC/MS). HPLC and GC/MS methods are also available for a major metabolite of DZ, nordiazepam.

Extraction of DZ and NDZ is often accomplished by solid phase extraction (SPE) or solid phase micro extraction (SPME), although liquid-liquid extraction methods are acceptable. The purpose of the current work is to develop a simple, rugged, sensitive, and specific method for the determination of DZ and NDZ using GC/MS. Midazolam is a structurally related benzodiazepine that was used as an internal standard.

In the current work, a method for the quantitative measurement of DZ and NDZ was developed. Human plasma samples were spiked with internal standard, adjusted to alkaline pH and extracted with ethyl acetate prior to detection by GC/MS.

Method: To human thawed plasma (1 mL) was added 40 µL of midazolam internal standard solution (400 ng/mL in H_2O) and various amounts of DZ and NDZ. Plasma samples were adjusted to pH 9 by the addition of 2N-NaOH and were extracted with 5 mL of ethyl acetate. The volatile components were removed in a stream of nitrogen. The residues were reconstituted in 50 µL of ethanol. The injection volume for GC/MS analysis was 2 µL. Sample preparation time was less than 30 minutes.

GC/MS analysis was performed using an Agilent MSD 5973 mass spectrometer operated in the electron-impact mode equipped with an injector operating in the splitless mode (with a 0.75-min splitless period) and the column was a DB-5MS capillary column (30 m x 0.25 mm x 0.25 ?m) using helium as carrier gas. The quantification was performed in the selected ion monitoring (SIM) mode using the most intensive three ions of the two compounds *(m/z* 256, 283 and 284 for DZ and *m/z* 242, 241 and 269 for NDZ).

The detection limit was 0.5 ng/mL and the assay was sensitive to 1ng/mL and linear to 100 ng/mL with correlation coefficients of > 0.999 for both DZ and NDZ. The recoveries of DZ and NDZ were 79%. The within-run CVs for DZ were 4.38%, 4.54% and 5.50% at 5.0, 20.0 and 60.0 ng/mL, respectively. The NDZ within-run CVs were 4.80%, 4.16% and 7.14% at the same concentrations, respectively. At these concentrations, between-run CVs for DZ and NDZ were less than 7.1% and 4.7%, respectively.

This sensitive and simple method is useful for plasma samples of forensic toxicological interest and in clinical studies when low concentrations of DZ are to be detected. Preliminary studies extended this approach to additional benzodiazepines. The results suggest that sensitive assay methods that do not require derivatization can be developed for midazolam (using an alternate internal standard), prazepam, and flurazepam. The method appeared to be less well suited for the development of methods for oxazepam, temazepam, lorazepam, flunitrazepam, alprazolam, and triazolam.

Diazepam, GC-MS, Method

K4 Influence of Ethylene Glycol Ethers and Other Organic Solvents on Quantitative Analysis of Breath Alcohol and an Introduction to Toxicology of 2-Butoxyethanol

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The participants will learn about the possible role of ethylene glycol ethers as interferents in evidentiary breath alcohol (BrAC) analysis and the toxicology related to ethylene glycol ethers.

Rules, statutes and *per se* laws provide for measuring ethanol concentrations in one of three principal media, *viz.,* blood, breath, and/or urine, with the ultimate goal of establishing the degree of intoxication either at sample collection time or another time. Breath analysis is the most frequently used method for the above purpose due to simplicity of sample collection and analysis. Breath analysis for ethanol is performed with the aid of IR-based instruments such as Intoxilyzer 5000EN®, the model used in the State of Minnesota for the purpose of evidentiary breath tests. BrAC measured by Intoxilyzer 5000EN® are often challenged in legal proceedings for many reasons including the presence of interferents in the breath analyzed for evidentiary purposes. These interferents are blamed for part or all of the BrAC measured in such occasions.

In a previous study, ~40 organic solvents and solvent formulations were tested for their possible role in erroneous measurement of BrAC by Intoxilyzer 5000EN®, and 12 substances (≤0.05 g/dL) that can increase BrAC by 0.01g/210L or more without being detected as interferents were identified. We have since tested an additional 5 compounds for their ability to significantly increase BrAC. These solvents, *viz*., 2-methoxyethanol (ME), 2-methoxyethyl acetate (MEA), ethylene glycol (EG), propylene glycol (PG) and diethyl ether (DEE), were chosen due to their presence in household products. Simulator solutions containing various concentrations (0.00001-10 g/dL) of the above substances were prepared by dissolving them in *aq* 0.10g/dL ethanol solution. The simulator solutions were subjected to the Minnesota breath test sequence in duplicate and on two different Intoxilyzer 5000EN® instruments as described previously to determine whether the simulated breath tests are terminated due to interferent detection. Air and *aq* 0.1g/dL ethanol served as negative and positive controls, respectively.

The concentration of interferent required for Intoxilyzer 5000EN® to automatically terminate breath tests were 0.1, 0.05 and 0.00005 g/dL for ME, MEA and DEE, respectively. The highest concentration of ME, MEA and DEE that did not trigger an automatic termination of breath tests were 0.09, 0.04 and 0.00004 g/dL, respectively. At these concentrations, the increase observed in BrAC was <0.01 g/210 L and was considered insignificant as per the criteria established in the previous study. EG and PG, on the other hand, did not terminate the breath tests or increase the BrAC even when their concentrations exceeded 10 g/dL.

Ethylene glycol ethers [(*e.g*., ME, MEA, 2-butoxyethanol (BE)], in general and BE in particular is of interest, because (i) the Intoxilyzer 5000EN® does not detect its presence in *aq* ethanol solutions (0.05-0.4 g/dL) until the concentration of BE >0.05 g/dL and at such concentrations BE accounts for a BrAC of ~0.04 g/210L, (ii) About 700 million pounds BE is manufactured, and used in industrial and household products (concentrations range from 2-25%) per year, (iii) NOISH estimates ~2.6 million American workers belonging to ~222 different occupations, *e.g.,* janitors, dry cleaners, printing machine operators and automobile mechanics, are exposed to BE and other ethylene glycol ethers every year, (iv) the ultimate metabolic product of BE, 2-butoxyacetic acid (BAA), is responsible for the toxicity of BE, and (v) it is unknown whether the metabolites of BE also interfere with breath analysis by Intoxilyzer 5000EN®.

The human toxicities associated with BE include delayed encephalopathy (8-12 hr) characterized by agitation, confusion, and/or coma, and metabolic acidosis (when consumed orally). Acute exposure of experimental animals to BE is associated with hemolysis and hemoglobinuria, hypocellular bone marrow, renal tubular degeneration, and necrosis. For these reasons, OSHA suggested limits of human exposure to BE is 25 ppm or lower. Although BE and other ethylene glycol ethers are available for systemic uptake by all routes, pulmonary and dermal routes of absorption represent the most significant and potential routes of human exposure at workplace. For example, the pulmonary retention of BE is ~57% in human volunteers. The concentration of BE detected in blood upon inhalation (air concentration 200 ppm; exposure time 3 hr) has been reported to be 0.0008 g/dL. The half-life of BE has been estimated to be 0.75-3 hr.

Using whole animal models, the metabolism of BE has been defined to resemble ethanol metabolism, *i.e.*, BE is first converted to 2-butoxyacetaldehyde (BAL) and then to BAA by alcohol and aldehyde dehydrogenases, respectively. Since BAA is more toxic than BE, then formation of BAA from BAL catalyzed by aldehyde dehydrogenases (ALDHs) seems to be the rate limiting step for toxicity due to BE. Human ALDHs exhibit allelic (isoforms) as well as non-allelic (isozymes) polymorphisms. The role of these polymorphic ALDHs in conversion of BAL to BAA is not known either in animals or in humans. In this regard, we have initiated research to identify the ALDH isozymes responsible for oxidation of BAL to BAA. BAL required for these studies was synthesized via Swern oxidation of BE. Human ALDHs, *viz.,* ALDH1A1, ALDH2 and ALDH3A1, required for this study were purified as described previously. Human cDNA clones overexpressing ALDH1A1 and ALDH2, respectively, in *E. coli* were the sources for ALDH1A1 and ALDH2, and human stomach mucosa was the source for ALDH3A1. Oxidation of BAL to BAA by ALDHs was determined as described previously using BAL as substrate. Each of the above ALDHs catalyzes the oxidation of BAL to BAA. ALDH2, the mitochondrial ALDH primarily responsible for oxidation of ethanol derived acetaldehyde (Km <1 µM) in humans, exhibits a Km of ~40 µM for BAL. ALDH3A1, the cytosolic ALDH mainly responsible detoxification of lipid peroxidation aldehydes, exhibits a Km of ~300 µM for BAL. Isolation of other ALDHs, determination of their affinity to BAL and their relative contribution towards the oxidation of BAL is on going. Whether the circulatory metabolites of BE, *e.g.,* BAL and BAA, interfere with quantitative analysis of ethanol, as in the case of BE, by Intoxilyzer 5000EN® is also being evaluated.

Based on the data collected thus far and given the tight regulations and limits of exposures imposed by OSHA, it is unlikely that any of the compounds we have tested will ever be found in breath samples at concentrations that significantly affect BrAC without being detected by Intoxilyzer 5000EN®. However, BE and other solvents are known to be abused. Whether, such scenarios lead to erroneous BrAC measurements is yet to be demonstrated.

Breath Alcohol Interferents, Toxicology of 2-butoxyethanol, Aldehyde Dehydrogenase

K5 Decomposition of Opiate Analgesics in the Presence of Formaldehyde

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The objective is to provide the forensic community with data describing the compound, pH and formaldehyde dependent decomposition of various opiate analgesics and the time course of this decomposition.

Background and Hypothesis: Opiate analgesics are commonly used in the treatment of pain, particularly severe pain, and are quite effective for this purpose. However, due to their potential to cause dependence, they have also been abused and thus have become drugs of forensic and toxicological interest since they may be involved in accidental or intentional deaths due to overdose. Thus, it is important to understand the chemical fate of these compounds since it may become necessary to assay for their presence at autopsy, following embalming or in preserved tissues. These latter two situations are the focus of the present work.

Formaldehyde is generally the primary component of embalming fluids and is also the standard chemical used to preserve tissue following autopsy. It is not uncommon to either require additional forensic toxicology testing either post-embalming or from tissue preserved from autopsy when foul play is suspected or the death appears suspicious following later evaluation of the evidence. However, formaldehyde is a highly reactive compound and as such possesses a quite complex chemistry. Previous work suggests that formaldehyde reacts with other drugs such as the tricyclic antidepressants, benzodiazepines, and fenfluramine to form new chemical products and thus hasten their decomposition. Thus, it was hypothesized that formaldehyde would also react with the opiate analgesics in an analogous manner and promote their decomposition which could complicate forensic analysis.

Methods: Codeine, morphine, hydromorphone, meperidine, methadone, oxycodone, nalorphine, and pentazocine were reacted with 5, 10 or 20% formaldehyde solutions at pH 3.5 (no pH adjustment of the solution), pH 7 and pH 9.5 to simulate the range of conditions to which tissues might be exposed either following embalming or upon preservation. Samples were analyzed by high performance liquid chromatography (HPLC) with UV detection for each of the opiates and time 0, 1 day, 7 days and 30 days, post-initiation of the reactions.

Results: Oxycodone decomposition was the most rapid of all the opiates with it achieving greater than 95% decomposition under pH 7 and 9.5 conditions at day 1 regardless of the formaldehyde concentration. Hydromorphone decomposed rapidly with greater than 70% decomposition noted at day 1 at both pH 7 and pH 9.5 regardless of formaldehyde concentration. Morphine decomposition was observed at pH 7, reaching ~50% decomposition at day 30, whereas at pH 9.5 almost 50% decomposition was noted at day 1 and approaching 100% decomposition at day 30 with the rate dependent on formaldehyde concentration. Nalorphine was relatively stable at pH 3.5 and pH 7, with only ~30% decomposition noted at pH 7 at day 30 but under pH 9.5 conditions, almost 100% of nalorphine had decomposed by day 30 with no difference among the various

formaldehyde concentrations. Methadone decomposed rapidly at both pH 7 and pH 9.5, both reaching essentially 100% decomposition at day 30 but the rate being faster at pH 9.5. Codeine, meperidine, and pentazocine did not appear to decompose over the 30 day time course regardless of pH or formaldehyde concentration with less than 10% decomposition noted under any of the conditions.

Conclusions: There appears to be compound dependent, pH dependent, and to some degree, formaldehyde concentration dependent decomposition of the opiate analgesics in formaldehyde solutions. These findings are of forensic interest in that they may establish a time profile for compound decomposition so as to permit determinations of whether drugs should still be present or whether alternate analytes might be appropriate.

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Opiates, Formaldehyde, Decomposition

K6 Low Level Detection of THC-COOH Using Negative Ion Chemical Ionization (NICI) GC/MS

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An analytical procedure for the low level detection of 11-nor-Δ^9-THC-9-carboxylic acid (THC-C00H) is described using a Varian 1200 Triple Quad Mass Spectrometer.

The use of various specimen types in forensic toxicology has made the determination of THCCOOH at low levels increasingly necessary in order to detect marijuana use. Instruments operating in chemical ionization modes (both negative and positive) coupled with selected ion monitoring and/or MS/MS are now widely available and affordable for the laboratory, although the number of published procedures using these methods is much lower than those using traditional electron impact modes of fragmentation. A sensitive and specific method has been developed for the quantitative assay of THC-C00H using a triple quadropole mass spectrometry system. The system can be operated in selected ion mode or mass spectrometrymass spectrometry (MS/MS) in order to improve the sensitivity of detection. A common disadvantage of chemical ionization, the production of a single m/z ion, is eliminated by the use of MS/MS The single ion is further fragmented to a characteristic “daughter” ion allowing for absolute identification of the analyte.

A Varian 3800 GC with a septum equipped programmable injector (SPI) was employed. The column was a 15m RTx 5 connected to a CombiPal autosampler and a Varian 1200 triple quadropole mass spectrometer. The initial injector temperature was 90°C with a temperature ramp at 100°C/min to 300°C. The initial column temperature was 90°C, held for 2 minutes then ramped at 25°C to 300°C. The carrier gas was helium, pumped at a constant flow of 1.4 mL/min. The transfer line was set to 270°C and the mass spectrometer was tuned in negative ion chemical ionization mode using PFTBA (calibration gas) for the tuning compound and methane was used as the reagent gas. The chemical ionization (CI) pressure was 7.8 x 10^{-4} Torr. The first quadropole (Quad 1) was set in the selected ion monitoring (SIM) mode collecting 4 ions; 422.3, 425.3 (d_3), 590.3 and 593.3 (d_3).

THC-COOH and deuterated THC-COOH-d_3 were evaporated to dryness and derivatized using trifluoroacetic anhydride (TFAA, 40 µl) and 1,1,1,3,3,3 hexafluoroisopropanol (HFIP) (20 µl). The mixtures were heated at 80°C for 45 minutes, then re-evaporated to dryness under vacuum, and reconstituted in toluene for injection into the MS system.

The following concentrations of THC-COOH were prepared: 100pg/µL, 10pg/µL, 1pg/µL, and 0.1pg/µL. Using the SPI injector, 1µL injections were made to determine limits of detection for the NICI GC/MS method on the Varian 1200 triple quadrupole. The lowest concentration tested (0.1 pg/µL) was readily detected, and repeated injections showed reproducibility and linearity over the range of 0.1-100pg/µL using a 1µL injection.

Triple Quadropole Mass Spectrometer, THC-COOH, MS/MS

K7 Metabolic Profile of Amphetamine and Methamphetamine Following Administration of the Drug Famprofazone

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The attendee can expect to learn the profile of the metabolically produced amphetamine and methamphetamine following the ingestion of famprofazone.

Several drugs lead to the production of methamphetamine and/or amphetamine in the body that subsequently excreted in the urine. These drugs raise concerns when interpreting positive amphetamine drug testing results. Famprofazone an analgesic is found in a multi-ingredient medication used for pain relief (Gewodin, Geistlich). Famprofazone is not available in the United States, but is available over-the-counter in European countries such as Germany.

Two tablets (50 mg of famprofazone) were administered orally to five volunteers with no history of amphetamine, methamphetamine, or famprofazone use. Following administration, urine samples were collected ad lib for up to seven days and pH, specific gravity, and creatinine values determined. To demonstrate the excretion profile of amphetamine and methamphetamine, samples were analyzed using liquid/liquid extraction, derivatized with heptaflurobutryic anhydride, and analyzed by gas chromatography/ mass spectrometry (GC/MS).

Peak concentrations for amphetamine ranged from 148 to 2,271 ng/ml and for methamphetamine 614 to 7,361 ng/ml. Concentrations of both compounds peaked between 3 to 14 hours post dose. Amphetamine and methamphetamine could be detected (LOD= 5 ng/ml) 121 hours and 143 hours post dose, respectively. Using a cutoff of 500 ng/ml, all subjects had individual urine samples that tested positive. One subject had 14 samples that were above the cutoff. The last positive being detected over 48 hours post dose.

Interpretation of results is a critical part of forensic drug testing due to potential repercussions to an individual. As demonstrated by the current study, a positive amphetamine test does not necessarily indicate illicit drug use. Evaluation of results with regard to those found in this study will assist in determination of the possibility of use of this medication as a source of methamphetamine and amphetamine.

Famprofazone, Amphetamine, Methamphetamine

K8 Effect of Freezing and Thawing on the Concentration of Ethanol in Urine Samples Stored in Leak-Proof Plastic Bottles

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The participants will learn how freezing and thawing affects concentration of ethanol in urine samples stored in leak-proof plastic bottles.

Social use of ethanol and its abuse continues to be important from the viewpoint of clinical and forensic interest. For forensic purposes, State and Federal legislatures, including Minnesota, have crafted rules, statutes, and *per se* laws to provide for measuring ethanol concentrations in one of

three principal media, *viz.,* blood, breath, and/or urine. The ultimate goal of measuring ethanol concentrations is to establish the degree of intoxication either at the time of sample collection or another time. These provisions often limit the choice to a single evidentiary sample and ethanol concentrations in the other samples may need to be derived. If the choice of evidentiary sample is urine, the above interpretation would be difficult but not impossible. Accordingly, the accuracy of measurement of ethanol concentrations in evidentiary samples and their interpretation, especially in the case of urine, are extremely important.

The accuracy of measurement and interpretation of ethanol results may be compromised for many reasons. For example, presence of relatively high concentrations of sugar, contamination of such samples by ethanol producing/metabolizing bacteria and/or yeast, *e.g.*, *E. coli* and *C. albicans*, and their storage at room temperature has been shown to result in *in vitro* ethanol production. This may be prevented by the addition of sodium fluoride (10 mg/mL) to samples at the time of collection and by storing them at 0-4°C prior to analysis. The above situation may also result from improper storage methods and procedures, especially in the case of urine samples. The laboratory at MN BCA, like most other laboratories, routinely stores evidentiary urine samples at 4°C prior to ethanol analysis, and frozen at -20°C post-ethanol analysis until they are (a) tested for drugs of abuse, (b) returned to the agencies that collected them, (c) transferred to dependents for re-testing when the results are disputed, or (d) destroyed when the samples are no longer required. Experience showed that re-testing the previously frozen urine samples for ethanol leads to the determination of ethanol concentrations that are significantly lower than those obtained prior to them having been frozen. This study was undertaken to establish the basis for lowering of ethanol concentrations in post-frozen urine samples and suggest corrective measures to prevent it.

Samples used for this purpose were (i) urine samples spiked with known concentrations of ethanol (n=100) and stored frozen for up to one year, (ii) actual case samples designated for disposal and stored frozen for up to two years (n=345), and (iii) urine samples collected from subjects participating in controlled drinking studies (n=38) and stored frozen for up to one year. Ethanol concentrations in these samples were quantified by head-space gas chromatography and the concentrations were expressed as g/67 mL as per Minnesota State Statutes. The containers used for this purpose were 100 mL leak-proof plastic bottles containing 1g NaF, routinely used for the collection of evidentiary urine samples in the State of Minnesota.

The frozen urine samples were either allowed to thaw slowly at 4°C in a walk-in cooler or allowed to thaw quickly at room temperature (RT) in a chemical hood. The method of thawing did not influence the magnitude of loss of ethanol concentrations in urine samples. The before freezing and after thawing ethanol concentrations (mean ± SD) in urine samples (n=90) thawed at 4°C were 0.15±0.048 and 0.10±0.058, g/67 mL, respectively. The before freezing and after thawing ethanol concentrations (mean ± SD) in urine samples (n=255) thawed at RT were 0.15±0.048 and 0.10±0.05, g/67 mL, respectively. Decrease in ethanol concentrations were not significant in urine samples (n=38) stored at 4°C (without ever being frozen) for zero (0.071±0.014 g/67 mL), 1 (0.071± 0.013 g/67 mL), 3 (0.072±0.012 g/67 mL) and 6 (0.070± 0.015 g/67 mL) months. Concentration of ethanol in the urine samples had little or no effect on the magnitude of ethanol lost during the above thawing process. For example, ethanol concentrations in urine samples (n=10; volume = 60 mL in each case) spiked with 0.05, 0.10, 0.15, 0.30 and 0.40 g/67 mL, frozen for one month and thawed at RT were 0.039±0.008, 0.081±0.007, 0.11±0.011, 0.23±0.025 and 0.32±0.03 g/67 mL, respectively. Ethanol concentrations in a second set of these samples thawed at 4°C (n=10; volume = 60 mL in each case) were 0.038±0.008, 0.080±0.008, 0.10±0.015, 0.24±0.030 and 0.31±0.04 g/67 mL, respectively. On the other hand, the decrease in ethanol concentrations observed above were dependent on the sample volume present in leak-proof plastic bottles used for their storage before analysis (4°C), during freezing (-20°C) and after thawing (at RT or 4°C). This correlation obtained for samples thawed at RT is shown in the table.

	AC (Mean ± SD), g/67 mL (n)		
Urine, mL	**Before Freezing**	**After Thawing**	**% Diff**
1-10	0.15±0.03 (7)	0.14±0.03 (7)	7.9±5.3
11-20	0.18±0.04 (9)	0.16±0.04 (9)	11±11
21-30	0.15±0.05 (17)	0.14±0.05 (17)	8.3±9.4
31-40	0.14±0.04(11)	0.12±0.04 (11)	9.8±19
41-50	0.13±0.04 (17)	0.12±0.04 (17)	7.2±11
51-60	0.15±0.04 (22)	0.12 ±0.04 (22)	18±23
61-70	0.13±0.05 (19)	0.09±0.04 (19)	23±20
71-80	0.15±0.05 (29)	0.11±0.05 (29)	26±20
81-90	0.15±0.04 (37)	0.10±0.04 (37)	32±23
91-100	0.16±0.05 (87)	0.08±0.04 (87)	47±26

Ethanol concentrations in urine samples were unaltered when they were frozen and thawed (at RT) in air tight vacutainer tubes (n=38; volume = 6 ml each; frozen for one year); the original and after thawing ethanol concentrations of such urine samples were 0.069±0.014 and 0.071±0.015 g/67 mL, respectively. Ethanol concentrations of the above samples (n= 38; volume = 60 ml each) processed identically but stored in leak-proof plastic bottles were 0.067±0.014 and 0.048±0.01 g/67mL, respectively. These studies suggest that the decrease in ethanol concentrations observed in post-frozen urine samples is due to improper sealing (likely due to decreased flexibility of plastic at -20°C) between the rim of plastic bottle and inner lining of its cap. The leak-proof 100 mL plastic bottles used for urine evidence collection contain an inner Styrofoam integrity seal attached to their mouth and complete removal of this seal before sample collection cannot not be easily accomplished. Further, during the process of thawing of frozen urine samples (ethanol does not freeze at -20°C), ethanol evaporates and escapes from the bottle via the small gaps/pores created due to improper sealing and thus leads to significant decrease in the ethanol concentrations. The pronounced loss of ethanol from the bottles containing larger volumes of urine samples is most likely due to expansion during freezing leading to disruption of Styrofoam seal and efficient evaporation of ethanol from smaller head space volume. Support for this notion comes from the observation that the ethanol concentrations of urine samples (n=19; sample volume 10-100 mL) before freezing (0.11±0.065), and after freezing (up to three months) and thawing (0.11±0.061 g/67 mL) were identical when the storage containers were essentially identical leak-proof 100 mL plastic bottles except that they did not have inner Styrofoam integrity seal. Accordingly, we have recommended and successfully adopted the use of leak-proof 100 mL plastic bottles (Tri-Tech, Inc., NC) that do not contain the inner Styrofoam seal, but contain an outer and across the cap tape integrity seal.

Ethanol, Urine, Head-Space Gas Chromatography

K9 DWI Cases Involving Anesthetics

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The objective is to illustrate the utility of using multiple GC columns with dissimilar retention characteristics for identification of unusual and unsuspected volatile substances in Driving While Intoxicated (DWI) cases.

During blood and urine headspace gas chromatographic analysis for ethanol in suspected Driving While Intoxicated (DWI) cases, toxicologists also routinely screen for acetone, methanol, and iso-propanol. Additional substances are sometimes observed and identified whenever possible. Minnesota statutes have criminal provisions for driving while under the influence of "hazardous substances" including solvents and other volatiles, as well as controlled substances. In most instances the observation of a volatile other than ethanol is germane to the driving behavior and the criminal case; however, occasionally it is not relevant. Cases illustrating both situations will be presented.

Analytical Procedure: Routine samples are diluted with a solution containing NaF and n-propanol (internal standard). Samples being examined for volatiles other than ethanol, methanol, isopropanol, or acetone may be analyzed undiluted as well. Analysis is via Perkin-Elmer Autosystem XL chromatographs containing capillary columns (either Restek BAC-1 or Restek BAC-2), or a Perkin-Elmer 8420 chromatograph containing a packed column (5% Carbowax 20M on Carbopack B); all instruments are equipped with Perkin-Elmer headspace samplers. When only ethanol is suspected, the isothermal analysis takes 4-5 minutes per sample. If other substances are suspected, the analysis may be extended to 20 minutes or longer. Subject samples are chromatographed on at least two dissimilar columns to detect the possible co-elution of substances on a particular column, and thus to establish the unique identity of the chemical observed in each peak. Relative Retention times (RRT) have been measured for a wide variety of compounds on these columns. A spreadsheet program aids identification of "unknowns" by calculating expected retention times (RT) for all known compounds when the RT of the internal standard is entered. Among the compounds examined were:

Compound	RRT(BAC-1)	RRT(BAC-2)	RRT(Carbowax 20M)
Methanol	0.638	0.411	0.326
Ethanol	0.707	0.533	0.498
Isoflurane	0.805	0.611	unk.
Diethyl Ether	0.807	0.396	0.421
Isopropanol	0.811	0.627	0.692
Acetone	0.937	0.578	0.432
n-Propanol	1.000	1.000	1.000

Case 1: A urine sample arrived by mail requesting "test for: cannibis (sic), inhalants, depressants." It was noted that no valid breath test was possible since the Intoxilyzer 5000 had detected an interferent. The subject had been examined by a Drug Recognition Expert (DRE) whose report mentioned several other possible intoxicants including ether, cyproheptidine, and a brand of cough syrup. Routine chromatography using the 5% Carbowax 20M column initially demonstrated two small peaks corresponding to ethanol and acetone. However, chromatography using the BAC-1 and BAC-2 columns demonstrated three peaks: a small "unknown" peak, as well as a trace (less than 0.01 g/100mL) of ethanol, and a trace of acetone. The third substance was identified as diethyl ether by its RTs on the BAC-1 and BAC-2 columns. Diethyl ether co-chromatographs with acetone on the Carbowax 20M column.

Additional drug of abuse screening was positive for 11-nor-9-carboxy-delta-9-tetrahydrocannabinol. Police reports furnished the additional information: PBT results at the scene were negative for breath alcohol. The subject admitted to smoking marijuana, and his breath had the odor of marijuana. He admitted previous abuse of inhaled chemicals (starter fluid) but denied use during the prior week. However, he had been observed by a reliable witness inhaling a chemical two days prior. The urine sample was obtained approximately 7.5 hours after the subject was apprehended.

Case 2: The same subject was stopped for DWI again one month later. He was immediately recognized and examined by the same DRE officer who had been involved in Case 1. Breath testing with the Intoxilyzer 5000 failed (instrument range exceeded). A blood sample was drawn approximately two hours after the subject was apprehended and submitted for "alcohol and ether" analysis. Chromatographic analysis of the blood showed a large peak with the RRT of diethyl ether, and a trace of acetone, but no ethyl alcohol. No other drugs or intoxicants were suspected or tested for.

Police reports furnished the additional information: PBT at the scene gave the false result of 0.135 (as ethanol). The subject's breath had a strong chemical smell (ether). The subject admitted to "huffing up" using Prestone Premium Starting Fluid (contains ethyl ether and hydrocarbon distillates) which was found in his car, and stated that he usually went through a 10 oz. spray can daily.

Case 3: A blood sample arrived by mail appearing to be a routine DWI; no drugs or other intoxicants were mentioned. Analysis revealed a prominent peak of an unknown substance, but no ethanol. Its RTs on the Restek BAC-1 and BAC-2 columns differed from any volatile we had previously examined. However, literature values suggested the possibility of the anesthetic Isoflurane. It was later discovered that the sample had been obtained while the subject was in the surgical operating room. A known sample of Isoflurane was obtained and shown to chromatograph identically with the substance in the subject's blood.

Conclusion: The observation of an "unusual" volatile substance, diethyl ether in Cases 1 and 2, was significant in their prosecution. The surprising observation of isoflurane in Case 3 was later determined to be irrelevant to its prosecution. Identification of these substances was possible through gas chromatography on multiple columns with dissimilar retention characteristics.

DWI, Volatiles, Chromatography

K10 Comparison of Drug Concentrations in Postmortem Cerebrospinal Fluid and Blood Specimens

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The objective is to gain insight into the usefulness of cerebrospinal fluid (CSF) for detecting different classes of drugs in postmortem analysis.

The ability to detect drugs of abuse in CSF specimens, such as opiates, has been well documented. This poster will present data which demonstrates that postmortem CSF specimens may be useful in the detection of a wide range of drugs as well.

The concentrations of drugs and their metabolites in cerebrospinal fluid and blood were determined in 282 autopsied cases using liquid-liquid extraction techniques and gas chromatographic analyses. All drugs were confirmed in one matrix by GC/MS. Blood was collected from the heart and placed in screw cap polyurethane tubes containing sodium fluoride (200 mg) and potassium oxalate (400 mg) and refrigerated at 4 °C. CSF was collected by cisternal puncture and stored in Vacutainer™ tubes containing 25 mg of sodium fluoride and 20 mg of potassium oxalate. CSF specimens were stored at –20 °C and were not tested until the result of the blood screen was known. For summarizing the results, the drugs were separated into different classifications with each class being represented by the major drugs found.

Some of the drugs found in the CSF specimens had a very wide range of CSF/blood ratios which skewed the average. For these drugs the median is also reported and tends to be a better representation of the values encountered. For example, amitriptyline ratios ranged from 0.03 – 2.93 with an average of 0.30 and a median of 0.10.

Benzodiazepines were detected in 55 of the 62 CSF specimens for which the blood was positive. Diazepam (n=43) and its major metabolite nordiazepam (n=51) accounted for most of the cases with average CSF/blood ratios of 0.36 (median =0.17) and 0.37 (median =0.21), respectively. Alprazolam was found in four cases with an average CSF/blood ratio of 0.85 (median =0.66).

The anticonvulsants, phenytoin (n=23), phenobarbital (n=8), and carbamazepine (n=5) were detected in 35 of the 36 cases analyzed. They had similar CSF/blood ratios of 0.33, 0.63, and 0.40, respectively.

Sedative drugs were represented mainly by carisoprodol (n=10) and meprobamate (n=21) and demonstrated higher average CSF/blood ratios than any other class of drugs (approx. 1.0). All the cases analyzed were positive.

Antihistamines were detected in 25 of the 37 CSF specimens analyzed with diphenhydramine (n=25) being the major drug with an average CSF/blood ratio of 0.34.

The narcotics, propoxyphene (n=21), methadone (n=8), and meperidine (n=7) all had CSF/blood ratios in the range of 0.3-0.4 and were detected in 32 of the 36 cases tested. Other narcotics found included tramadol and oxycodone.

Of the tricyclic antidepressants, amitriptyline (n=20) was the major drug found with an average CSF/blood ratio of 0.3 (median =0.1), while nortriptyline, the major metabolite, had a lower ratio of 0.06 and was only detected in half of the cases. Similar results were obtained for imipramine and desipramine. Some of the other antidepressants detected included bupropion, sertraline, fluoxetine, citalopram, and trazodone with CSF/blood ratios ranging from 0.02 – 0.4.

Anesthetic drugs found were lidocaine (n=45), phencyclidine (n=7), and ketamine (n=2). Lidocaine had high average CSF/blood ratio of 1.19 with a lower median ratio of 0.47. Phencyclidine had a lower ratio of 0.17.

Very few of the drugs that were analyzed were not readily detected in CSF specimens. The average CSF/blood ratios for most drugs was in the range of 0.05-0.50. Interpretation of these results are difficult since CSF drug concentrations will vary significantly with the amount of time between drug usage and the time of death. The half-lives, hydrophobic properties, and pKa's of the drugs will also influence the CSF/blood ratios. However, CSF specimens do provide a viable alternative testing matrix when blood specimens are not available but should not be used to estimate blood drug concentrations.

Cerebrospinal Fluid, Postmortem, Analysis

K11 Evaluation of the Protective Effect of N-acetylcysteine on the Lungs of Paraquat Intoxicated Rats

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The aim of the present study was to evaluate the protective effect of N-acetylcysteine on the lungs and liver of Paraquat intoxicated rats.

Paraquat is an herbicide widely used in agriculture. Death from paraquat intoxication has been reported both in humans and in experimental animals with involvement of lung, kidney, myocardium, central nervous system, liver, spleen, adrenals, and muscles. Progressive pulmonary damage occur due to irreversible changes in the pulmonary tissues which are not recognizable clinically until they are sufficiently advanced, leading to respiratory failure and causing most of the fatalities due to the selective concentration of paraquat in the lung parenchyma. It is now accepted that the toxicity of paraquat is based on an oxygen free radical mechanism. To counteract paraquat toxicity, induction of reactive oxygen species must be decreased. Different pharmacological strategies have been explored to reduce the formation of these reactive oxygen species and/or prevent their toxic effects in the treatment of paraquat poisoning. N-acetylcysteine (NAC) is a known antioxidant and free radical scavenger. The underlying mechanisms of its effects include its action as a precursor of the natural antioxidant glutathione, or to the direct reaction between the reducing thiol residue of NAC and the oxygen species formed under the influence of an oxidant, or from activated macrophages and neutrophils during inflammation. From this point of view the present study was carried out in order to evaluate the protective effect of NAC on the lungs and liver of paraquat intoxicated rats. Forty-five male albino rats (180-200 g) were used in the present study, The animals were divided into five groups (nine animals in each group):

(I) Control group; (11) NAC group; (111) Paraquat intoxicated group; (IV) Paraquat intoxicated NAC-treated group, given NAC at the same time with paraquat; and (V) Paraquat intoxicated NAC-treated group, given NAC 4 hr after paraquat intoxication. The intoxicated rats received 20mg/kg paraquat IP and were treated with NAC in a dose of 50mg/kg IP. Histological examination of the lung and liver sections was done using:

(1) Haematoxylin and Eosin stain (for routine histological examination);
(2) VVG stain (for elastic tissue); and
(3) Trichrome stain (for collagen fibers).

From our results, it could be concluded that intraperitoneal administration of NAC at the same time with paraquat as a prophylactic agent had no protective effect against paraquat induced lung and liver injury in rats. On the other hand, NAC when administered 4 he after paraquat intoxication as a curative agent did have an obvious therapeutic effect against paraquat toxicity in rats' lung and liver

Paraquat, N-acetylcysteine, Lungs

K12 Peak Δ9-Tetrahydrocannabinol (THC), 11-hydroxy-THC (11-OH-THC) and 11-nor-9-Carboxy-THC (THC-COOH) Excretion & Detection Times in Human Urine Following Controlled Administration of Smoked Marijuana

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After attending this presentation, the participant will be better informed on interpretation of THC, 11-OH THC & THC-COOH in urine following smoked marijuana

Marijuana and hashish, derived from Cannabis Sativa, produce physiological and behavioral subjective intoxication following cannabis smoking. The main psychoactive compound of Marijuana is THC, which is rapidly bio-transformed to an active metabolite, 11-OH-THC. Further oxidative metabolism forms the inactive, more polar metabolite, THC-COOH. THC-COOH and its glucuronidated metabolite are the major metabolites found in urine. In routine criminal justice, treatment and workplace drug monitoring programs, evaluation of marijuana exposure is usually accomplished by measuring THC-COOH in urine following alkaline hydrolysis. As suggested by Kemp et al (*JAT* 1995;19:292), enzymatic hydrolysis with E. coli Type IX bacteria permitted release and subsequent measurement of both active components, THC and 11-OH-THC. We have further developed this idea by combining an enzymatic hydrolysis with E. coli Type IX-A bacteria followed by an alkaline hydrolysis, solid phase extraction with a dual elution solvent and sensitive electron impact gas chromatography-mass spectrometry (GC/MS) analysis. We suggest that THC and 11-OH-THC will be present in the urine for a shorter period of time than THC-COOH, but their presence may provide important information on the recency of marijuana smoking, especially during excretion in heavy users.

This study examined the timecourse of THC, 11-OH-THC, and THC-COOH in urine following controlled administration of smoked marijuana. Subjects (N=11) were healthy volunteers with a history of marijuana use. The study was conducted under the guidelines for the protection of human subjects, and each volunteer gave written informed consent. During the study, all subjects resided on a closed research ward at the NIDA Intramural Research Program under medical surveillance to prevent self-administration of additional drugs. Each subject smoked a single 2.78% THC cigarette on two dosing sessions separated by seven days. All urine specimens were collected ad lib throughout the study. Urine was processed and frozen at –20°C until GCMS analysis. Prior to analysis, 2 mL of urine was hydrolyzed with 10,000 units of E. coli β-glucuronidase type IX-A for 16 hrs at 37°C. This was followed by base hydrolysis with 10N NaOH and heated at 60°C for 15 minutes. The hydrolyzed samples were processed by solid phase extraction (Clean Thru DAU, UCT), derivatized with BSTFA (1%TMCS). Mean peak THC concentration in the urine was 6.9 ± 2.3 ng/mL (range 0-42.8).. Peak THC concentration occurred within the first half-hour after marijuana smoking.

Mean peak 11-OH-THC concentration in urine was 42.1 ± 10.5 ng/mL (range 4.4-231.1) and occurred at 3.7 ± 0.7 hrs (range 0.5-10.9). Mean peak THC-COOH urine concentration was reached at 14.7 ± 1.7 hrs (range 5.6-39.1) and varied from 9.2 to 315.2 ng/mL (mean 81.4 ± 15.1).

In addition to the controlled drug administrations, the three cannabinoids were quantitated in urine specimens collected from the time of admission to the secure research unit until completion of elimination (wash-out period). The wash-out period ranged from 12.8-28.5 days for four subjects. Peak THC, 11-OH-THC, and THC-COOH concentrations occurred within the first four urine voids. THC was measurable (5.8 and 7.3 ng/mL) in two of the four participants, indicating recent marijuana smoking. Peak 11-OH-THC urine concentrations ranged from 11.0 to 104.0 ng/mL and occurred prior to 7.5 hrs. Peak THC-COOH urine concentrations ranged from 61.9 to 1081.7 ng/mL and occurred prior to 3.5 hrs.

Each of the eleven subjects participated in two controlled smoking sessions for a total of 22 sessions. The detection times of THC, 11-OH-THC, and THC-COOH in the controlled administration sessions were in the ranges indicated below..

Detection Times (hrs)	THC	11-OH-THC	THC-COOH
0 – 10 hrs	22	0	0
>10 – 24 hrs	0	4	1
>24 – 36 hrs	0	4	1
>36 – 48 hrs	0	4	2
>48 – 72 hrs	0	2	5
> 72 hrs	0	8	13

These preliminary data indicate that detection of THC in urine may be a good indicator of recent marijuana smoking; however, 11-OH-THC appears to be present in some subjects for more than 72 hrs, well beyond the period of intoxication, and may, therefore, be less useful in documenting recent exposure.

Smoked Marijuana, Controlled Administration, Urine

K13 Characteristics of Calibration Curve Resulting From the Use of a ^{2}H-Analog of the Analyte as the Internal Standard—Methamphetamine Example

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The objective is to familiarize the participants with the complicating factors affecting the linearity of the calibration curve resulting from the use of a ^{2}H-analog of the analyte as the internal standard (IS). Specific parameters studied include (a) reconstitution and injection volume that may affect the molecular abundance of the analyte and the IS in the ion source, and (b) column temperature programming conditions and the number of the ^{2}H-atom incorporated in the IS that may affect the separation of the analyte and the IS.

While evaluating the effectiveness of ^{2}H- and ^{13}C-analogs of the analytes in serving as the ISs in various barbiturate systems, it was noted that the intensity ratio of an ion-pair designated for an analyte and its ^{2}H-analog increases as the extraction-derivatization residue is reconstituted with increasing amount of solvent. Realizing the effects of this phenomenon on the characteristics of the calibration curve, this current study is designed to: (a) confirm the occurrence of this same phenomenon in the amphetamine drug category, (b) examine the effect of this phenomenon on the calibration curve, and (c) study factors underlying this phenomenon.

$^{2}H_{5}$-, $^{2}H_{9}$-, and $^{2}H_{14}$-analogs of methamphetamine were adapted as the ISs for calibration curve evaluation. Well-established solid-phase extraction and pentafluoropropionyl (PFP) derivatization procedures were used to pretreat standard solutions prepared in urine matrix. A series of standard solutions containing 100-9600 ng/mL methamphetamine and 500 ng/mL IS were used to examine the characteristics of the calibration data.

Calibration lines shown in Figure 1 clearly indicate the difference when the calibration line is established with different injection volumes. Apparently, the intensity ratios of the designated ion-pair at the higher concentration end derived from smaller injection volume are relatively higher. It is believed this is caused by the non-proportional decrease (relative to the IS) in the ionization efficiency of the analyte as its molecular abundance at the ion source increases.

To further study the molecular abundance issue, a second series of experiments was performed, in which 50, 150, and 450 mL ethyl acetate was used to reconstitute the extraction-derivatization residue. Data shown in Table 1 clearly demonstrate that, as the reconstitution volume increases (i.e., the molecular abundance at the ion source decreases), the ion intensity of the designated ion-pair at the higher concentration end stays closer to its "expected" value, i.e., the "linearity" of the calibration curve can be extended to a higher concentration level.

Thus, it was confirmed that the methamphetamine/$^{2}H_{14}$-methamphetamine system exhibits the same characteristics as the barbiturate/$^{2}H_{5}$-analog systems. Since parallel barbiturate/^{13}C-analog systems are free of this interference phenomenon, it was hypothesized that the retention time difference between the analytes and their ^{2}H-analogs is the underlying cause for the observed non-proportional decreases in ionization efficiency in the analyte/^{2}H-analog systems. (Analytes and their ^{13}C-analogs have identical retention time.) Thus, retention time difference is the parameter studied in the next series of experiments. Data shown in Table 2 clearly indicate that, as the analyte and its ^{2}H-analog IS are further overlapped (by programming at a higher rate), the designated ion-pair intensity ratios for the standards with higher analyte concentrations become closer to their "expected" values, i.e., the "linearity" of the calibration can be extended to a higher concentration level.

To further study the retention time difference parameter, another series of experiments are performed, in which $^{2}H_{5}$-, $^{2}H_{9}$, and $^{2}H_{14}$-methamphetamine (showing increasing retention time difference with the analyte) are used as the ISs. Resulting data shown in Table 3 indicate that, compared to the methamphetamine/$^{2}H_{9}$-analog and the methamphetamine/$^{2}H_{14}$-analog systems, the intensity ratio of the ion-pair designated for methamphetamine/$^{2}H_{5}$-methamphetamine at the higher concentration end is closer to their "expected" values. Again, this will allow the extension of the linearity of the calibration curve to a higher concentration level.

It has been demonstrated that establishing a calibration line within a desired concentration range, in addition to careful selection of the internal standard, requires careful consideration of the extraction-derivatization reconstitution volume, injection volume, and temperature programming parameters.

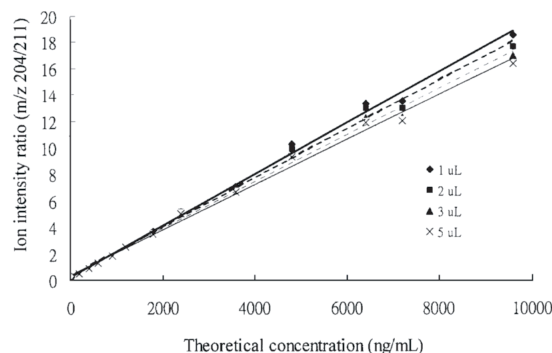

Figure 1. Effects of injection volume on calibration line characteristics–Methamphetamine/$^{2}H_{9}$-analog: m/z 204/211.

Table 1. Effect of reconstitution volume on the linearity of the calibration line—Methamphetamine/$^2H_{14}$-analog: m/z 204/211

Theor. Conc. (ng/mL)	50 µL reconstitute volume: Ion ratio	Dev. (%)	150 µL reconstitute volume: Ion ratio: Ratio change (%)†	Dev. (%)	450 µL reconstitute volume: Ion ratio: Ratio change (%)†	Dev. (%)
100	0.247	17.5	0.239: -3.23	16.4	0.250: 1.21	14.9
200	0.429	2.14	0.413: -3.73	0.59	0.434: 1.17	-0.17
400	0.841	Calibrator	0.822: -2.26	Calibrator	0.870: 3.45	Calibrator
600	1.246	-1.25	1.224: -1.77	-0.69	1.299: 4.25	-0.45
900	1.870	-1.16	1.828: -2.25	-1.14	1.967: 5.19	0.50
1200	2.403	-4.77	2.379: -1.00	-3.53	2.546: 5.95	-2.46
1800	3.629	-4.12	3.606: -0.63	-2.51	3.906: 7.63	-0.22
2400	5.119	1.44	5.140: 0.41	4.22	5.585: 9.10	6.99
3600	7.082	-6.44	7.135: 0.75	-3.55	7.825: 10.49	-0.06
4800	9.123	-9.60	9.373: 2.74	-4.98	10.278: 12.66	-1.55
6400	11.657	-13.37	11.974: 2.72	-8.96	13.385: 14.82	-3.84
7200	13.328	-11.96	13.601: 2.05	-8.08	15.197: 14.02	-2.96
9600	17.216	-14.70	17.757: 3.22	-9.99	19.633: 14.04	-5.97

† Ratio changes are calculated by dividing the ratio observed with the reconstitution volume of 50 µL by the ratio observed with the reconstitution volume of interest.

Table 2. Effect of variation in Methamphetamine/$^2H_{14}$-analog (m/z 204/211) retention difference (resulting from different temperature programming) on the linearity of the calibration line—$^2H_{14}$-analog: 400 ng/mL.

Recons. Vol. (µL)	Ion ratio: Ratio change (%)† 200 ng/mL methamphetamine		Ion ratio: Ratio change (%)† 3600 ng/mL methamphetamine		Ion ratio: Ratio change (%)† 9600 ng/mL methamphetamine	
	25° temp. ramp	5° temp. ramp	25° temp. ramp	5° temp. ramp	25° temp. ramp	5° temp. ramp
150	0.509	0.483	8.174	8.139	22.285	20.935
300	0.495:- 2.75	0.501: 3.73	8.334: 1.96	8.513: 4.60	23.576: 5.79	23.312: 11.35
450	0.505: -0.79	0.496: 2.69	8.606: 5.29	8.693: 6.81	24.395: 9.47	25.055: 19.68
600	0.498: -2.16	0.476: -1.45	8.758: 7.14	9.516: 16.92	25.399: 13.97	26.898: 28.48

† Ratio changes are calculated by dividing the ratio observed with the reconstitution volume of 150 µL by the ratio observed with the reconstitution volume of interest.

Table 3. Effect of variation in analyte/^2H-analog IS retention difference (resulting from the use of the ^2H-analogs with different number of ^2H-atoms) on the linearity of the calibration line—IS: 400 ng/mL.

Theor. Conc.	Methamphetamine/ $^2H_5$-analog (m/z 204/208)		Methamphetamine/ $^2H_9$-analog (m/z 204/211)		Methamphetamine/ $^2H_{14}$-analog (m/z 204/211)	
	Ion int. ratio	Deviation (%)	Ion int. ratio	Deviation (%)	Ion int. ratio	Deviation (%)
100	0.226	-1.79	0.307	10.02	0.263	11.24
200	0.467	1.51	0.572	2.43	0.475	0.61
400	0.921	Calibrator	1.117	Calibrator	0.945	Calibrator
600	1.398	1.17	1.703	1.64	1.378	-2.79
900	2.047	-1.23	2.613	3.96	1.993	-6.26
1200	2.782	0.69	3.324	-0.82	2.844	0.32
1800	4.215	1.69	5.035	0.16	3.988	-6.22
2400	5.646	2.17	6.540	-2.42	5.514	-2.76
3600	8.487	2.39	9.891	-1.62	7.979	-6.18
4800	11.23	1.59	12.69	-5.30	10.82	-4.56
6400	14.86	0.84	17.79	-0.45	14.64	-3.19
7200	16.81	1.37	20.00	-0.55	16.14	-5.11
9600	22.63	2.36	26.15	-2.46	22.16	-2.32

Internal Standard, Ion Intensity Ratio, Methamphetamine

K14 Detection of Gamma-Hydroxybutyrate (GHB) and Gamma-Butyrolactone (GBL) in Driving Under the Influence Cases Within the New Jersey State Police Forensic Science Bureau

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The objective is to present findings regarding the detection of Gamma-Hydroxybutyrate (GHB) and Gamma-Butyrolactone (GBL) in blood and urine from driving under the influence cases and describe the methodology used.

The New Jersey State Police Forensic Science Bureau has seen an increase in driving under the influence cases containing GHB and/or GBL. A summary of the results seen within the laboratory and the methodology used is presented. A procedure was established for screening and quantitation of GHB/GBL using an adaptation of the Headspace GC-FID method developed by the FBI Chemistry Unit.

Alpha-methylene-gamma-butyrolactone internal standard is added to two aliquots of biofluid. Concentrated sulfuric acid is added to one aliquot to convert any GHB present to GBL. A four point GHB calibration curve (10 µg/mL-100 µg/mL) is prepared and also treated with concentrated sulfuric acid. All aliquots are extracted with methylene chloride and concentrated. Extracts are screened and quantitated using Headspace GC-FID. Samples calculated to be greater than or equal to 15 µg/mL are confirmed using full scan GC-MS. The confirmation of GBL employs the alpha-methylene-gamma-butyrolactone internal standard and a simple methylene chloride extraction.

The GHB confirmation procedure utilizes the solid-phase extraction method developed by United Chemical Technologies, Inc., with modifications to utilize full-scan GC/MS. Blood samples containing GHB require a cleanup step with acetone. Urine samples containing GHB are treated with a urease solution to break down the urea into ammonia, water, and carbon dioxide. These samples are then spiked with GHB-D6 internal standard and buffered with phosphate buffer. Clean Screen® GHB extraction columns are used on the Zymark Rapid Trace™ SPE Workstation providing a rapid procedure for the extraction of GHB from blood and urine samples. The eluate is evaporated to dryness and reconstituted with ethyl acetate and BSTFA with TMCS. Full scan GC-MS (EI) confirmation of GHB is then obtained from the GHB-diTMS. This procedure provides sufficient sensitivity for the screening, quantification, and confirmation of GHB/GBL in toxicology cases involving driving under the influence.

Gamma-Hydroxybutyrate, Gamma-Butyrolactone, Toxicology

K16 Methyl Alcohol Intoxication Resulting in Death in Turkey: An Eight-Year Autopsy Study

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The objective is to establish the general specifications of methyl alcohol intoxication in Turkey.

The aim of this study was to examine methyl alcohol poisoning cases from the medico-legal point of view. The records of the morgue

department of the Council of the Forensic Medicine were reviewed retrospectively for all methyl alcohol poisonings for the period of 10-27-1992 to 05-30-2001. The victim's age, sex, date of death, death place, blood methyl alcohol levels, the source of methyl alcohol, accompanying laboratory results, and histopathologic tissue changes were recorded.

Blood samples were collected from the pericardial sac for the measurement of alcohol concentration. All samples were steam distilled with n-butanol (0.5 ml of 80 mg/dl) as internal standard before gas chromatographic analysis. Methyl alcohol analysis was performed on a Hewlett-Packard model 5890 series Gas Chromatograph-Flame Ionization Detector (FID) equipped with a Hewlett-Packard model 7694 Headspace Sampler. The injection port and detector temperatures were 200°C and 280°C, respectively.

According to the Forensic Medicine Morgue Department records, the total number of the deaths between 1992-2001was 24,206. The number of the deaths due to the methyl alcohol poisoning was 271(1.12%) during this period. Two hundred forty-two (89.3 %) of the total 271 methyl alcohol fatalities were men and 29 (10.7 %) were women. The ages varied from 16 years to 68 years. The largest group was 36-40 years old, followed by 41-45.

The number of methyl alcohol poisonings was increasing gradually with respect to the annual distribution from 1992 to 2001.

The blood methyl alcohol concentrations ranged widely from 50 to 755 milligrams per 100 ml. There were 222 cases (81.9 %) with the blood methyl alcohol concentrations over 100mg/dl. In six cases the victims were treated in different hospitals and they had blood methyl alcohol levels under 50 mg/dl. Formaldehyde was detected in 12 of these cases and 75 of them had also ethyl alcohol in blood samples. Two of them also had barbiturate derivatives in their blood and urine samples. Formic acid wasn't detected in any of the cases.

Twenty-nine (10.7 %) victims were poisoned through the consumption of cologne and three of them with an alcoholic beverage named "Rak?." Consumed products were not known in all other cases because of insufficient history and data. All of these cases were suspicious – 61 (22.5%) died in hospital, 103 (38%) died at home, and 44 (16.2%) on a road etc.

In Turkey, section 21 of the regulation Nr. 3/15481 about foodstuffs and goods and containers effecting the public health and hygiene prescribes: ethyl alcohol must be used in contents of all alcoholic beverages, must be used in all distillated alcoholic beverages and methyl alcohol mustn't be used in alcoholic beverage named "Rak?."

In conclusion, methyl alcohol deaths are gradually increasing and have a great significance in toxicological deaths. There were no specific histopathologic findings and the significance of the laboratory analysis to legal investigation was shown. Preventative strategies for the production and consumption of illicit alcoholic beverages must be developed.

Methyl Alcohol, Intoxication, Forensic Medicine

K17 Issues Related to Simulator Temperature Measurement in a Breath Alcohol Simulator

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The attendee will learn about the limitations of temperature measurement and stability in breath alcohol simulators, the impact of this on the breath test, and the forensic implications of thermometer record keeping in breath alcohol testing.

Breath alcohol test instruments are typically calibrated and checked for accuracy with vapor from an alcohol water solution at a known temperature, based on Henry's law. During the process of validating new software for the DataMaster breath test instrument (National Patent Analytical Systems, OH), it was discovered that a mercury-in-glass thermometer in at least one Guth 34C breath alcohol simulator (Guth Laboratories Inc., PA) was reading inaccurately. Further investigation revealed that as many as 25% of the custom made, NIST traceable thermometers, designed to measure accurately between 33.5 and 34.5 degrees centigrade, were inaccurate by as much as half a degree. This inaccuracy and its scientific and forensic significance has become the focus of extensive litigation in Washington State, resulting in suppression of hundreds of breath alcohol test results on grounds of failure to comply with appropriate regulations, rather than because of any inaccuracy of the breath test result itself.

During this litigation the authors conducted a review of the scientific literature on mercury in glass thermometers, performed experiments demonstrating the influence of the thermometer inaccuracy on the simulator ethanol result, used the field test database to examine the instrument's ability to accurately measure the ethanol concentration of the simulator solution compared to an independent gas chromatographic measurement, and examined the stability of the thermostat controlled simulator temperature over both the short term (20 minutes – 24 hours), and the longer term (up to 30 days).

Because of the multiple cross checks and other safeguards imposed on the breath test program by both administrative rule and by protocol, it was successfully demonstrated that the unreliability of the mercury-in glass-thermometers did not affect the accuracy of the test results.

The lessons learned from this experience were as follows:

- In contrast to popular belief, even NIST certified mercury-in-glass thermometers can lose calibration over time, and should be periodically checked.
- Inaccuracy in the mercury in glass thermometers had no effect on the accuracy of the breath alcohol test, a position that was not disputed in any subsequent litigation.
- The thermostat-controlled temperature of the Guth 34C simulator can exceed the specifications claimed by the manufacturer.
- Digital thermometers are not necessarily any more reliable than mercury in glass thermometers, and may in fact have greater limitations.
- Demonstration of scientific reliability may not be sufficient in a forensic setting; literal compliance with written rules must be checked and complied with.
- The wording of administrative rules is critical regarding the "interpretive compliance" of analytical protocols.
- The language of administrative rules defining test admissibility are best kept to a minimum with technical analytical details reserved for policy and analytical protocol manuals.
- Record keeping and disclosure are critical in all aspects of a forensic breath test program.

Breath Alcohol, Thermometer, Calibration

K18 Detection of Cortisol and Cortisone in Human Hair

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The participant will learn that cortisol end cortisone can be detected and quantified in human hair and that there is evidence for their integration through sweat.

Since the 1960s, corticosteroids have been used by athletes to improve their performances. Their use is restricted in sports. Hair can document chronic abuse and is therefore a complementary matrix to urine for doping control.

The authors have developed a new extraction, purification, and separation technique using liquid chromatography and mass spectrometry for the identification and quantification of two endogenous

corticosteroids: cortisol and cortisone. Forty-five hair samples (17 males, 28 females; age ranging from 2 to 90 year-old) were studied. Hair strands were washed in methylene chloride, the first two centimeters of the strand were cut and pulverized in a ball mill. The powdered hair was incubated in 2mL Soerensen buffer, pH 7.6 for 16 hours at 40°C, in the presence of cortisol-d_3 as an internal standard. Purification of the incubation medium was achieved on SPE C18 Isolute extraction columns followed by an alkaline liquid-liquid extraction with diethylether. The eluate was evaporated to dryness and resuspended in 25µL of acetonitrile/ammonium formate (1:1,v/v). The chromatography was operated on a LC Packings Superba® Nucleosil C18 column using a linear gradient of acetonitrile from 30 to 70% in 10 min. The detector was a Perkin Elmer Sciex API 100 mass spectrometer. The detector's response was linear for cortisol and cortisone concentrations ranging from 1 to 500pg/mg. Extraction recovery at 50pg/mg was 74% for cortisol and 32% for cortisone. Repeatability (CV values) evaluated on 1g homogenized hair and 7pg/mg cortisol was 11% and at 50pg/mg cortisone was 11%. The limit of detection was 1pg/mg and the limit of quantification 5pg/mg. Cortisol concentrations in hair ranged from 5 to 91pg/mg (mean 18 pg/mg). Cortisone concentrations in hair ranged from 6 to 163 pg/mg (mean 68pg/mg). No influence of hair color could be found. Influence of sex on cortisone concentrations seemed possible but could not be statistically demonstrated. Cortisone concentrations in hair are significantly higher before the age of twenty.

Type 2 11β-Hydroxysteroid-dehydrogenase (HSD) converts cortisol to cortisone, whereas Type 1 HSD, which is mainly present in the liver converts cortisone to cortisone. Type 2 HSD is present in cells where mineralocorticoid receptors are present (kidney, sweat, salivary glands, gastro-intestinal tracts). In human epithelia, Type 2 HSD has been located in sweat glands whereas it is absent from sebaceous glands and hair follicles. Also its activity is higher in children, protecting the child's growth against cortisol effects by converting cortisol to cortisone. The results show that cortisone concentrations are higher than cortisol concentrations in hair unlike blood ratio and that cortisone concentrations are significantly higher before the age of 20. It is known that cortisone is less polar than cortisol and therefore one would expect a better incorporation of cortisone than cortisol into hair from bloodstream. But as the blood ratio of cortisol to cortisone is not different between adults and children, the incorporation through bloodstream and the different polarity of cortisone and cortisol cannot explain why cortisone concentrations are significantly higher in children. This suggests a relationship between cortisone concentrations in hair and type 2 HSD. Incorporation of cortisol and cortisone in hair could follow a passive diffusion through sweat after conversion of part of cortisol to cortisone by Type 2 HSD in sweat glands.

Corticosteroids, Hair, Sweat

K19 Distribution of Selegiline Metabolites in Pigmented and Senile White Hairs From Parkinson Patients

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During this presentation the participants will learn about the influence of melanin on the incorporation of methamphetamine and amphetamine, major metabolites of the anti-Parkinson drug selegiline, in human hair. The data is important for the interpretation of forensic hair samples. Distribution of Selegiline metabolites in pigmented and senile white hairs from Parkinson patients. The objective of this study was to investigate drug concentrations in senile white hair and pigmented hair from the same patient separately. The study was approved by the regional ethics committee of the Faculty of Health Sciences, University of Linköping, Sweden.

Background: Considerable research effort has been directed towards the issue of bias associated with hair color in drugs of abuse testing. One way of studying the potential influence from melanin is to measure drug content in pigmented and non-pigmented hair from the same animal or human subject. Several animal studies have shown that weakly basic drugs such as cocaine, codeine, and amphetamine are preferentially incorporated into pigmented hair rather than non-pigmented hair. Also in studies on people with gray hair have shown that cocaine, haloperidol, and amitriptyline are found in significantly higher concentrations in pigmented hair than in senile white hair. The concentration profile in pigmented hair differs widely, probably because of different melanin content. It was previously shown that incorporation of methamphetamine into human hair depends on melanin content. Studying the distribution of the amphetamines in pigmented and senile white hair from the same individual will improve the means of correctly interpreting drug concentrations in hair.

Experimental: Hair samples were obtained from nine gray-haired patients with Parkinson's disease receiving Selegiline daily for at least three months prior to sampling. Dose, age, gender, hair color, weight, and height of the patients were recorded. Hair samples (triplicates) were obtained from the posterior vertex, and cut with scissors as close to the scalp as possible. The samples were folded in aluminum foil and stored in darkness at room temperature until analyzed. Hair was weighed in a screw-capped glass tube and internal standard was added (2.0 ng of each methamphetamine-d5, and amphetamine-d5). One mL of 1 M KOH was added and the hair sample was heated at 80° C for 10 minutes with occasional shaking.

After cooling to room temperature the sample was extracted with 4 mL of isooctane for 10 minutes, centrifuged at 4200 g for 5 minutes, before the isooctane was transferred to a clean tube and re-extracted into 0.5 mL 0.1M sulfuric acid. After centrifugation for 5 minutes at 4200 g the isooctane was aspirated and discarded and 0.5 mL of 1 M KOH was added.

Then 3 mL of isooctane was added and the sample was extracted for 10 minutes. After a 5 minute centrifugation at 4200 g the isooctane was transferred to a new 10-mL screw-capped glass tube and 20 µL of MeOH:HCl (99:1 v/v) was added. The sample was mixed and evaporated under a gentle stream of nitrogen at room temperature. When about 30 µL of the isooctane remained 50 µL of TFAA was added and the tube was capped and heated for 20 minutes at 60°C. After cooling to room temperature the sample was evaporated and reconstituted in 50 µL isooctane, and transferred to a GC-vial. GC-MS was used for analysis. The ions monitored were m/z 118.0 and m/z 140.0 for amphetamine, m/z 110.0 and m/z 154.0 for methamphetamine, m/z 118.0 and m/z 178.0 for desmethylselegiline. For the internal standards the ions monitored were m/z 144.0, m/z 158.0 for amphetamine-d5, and methamphetamine-d5, respectively.

A 13-point calibration curve was performed by addition the of the analytes to 20 mg drug-free hair (obtained from a laboratory employee). Calibration levels were at 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 1.0, 2.0, 4.0, 8.0, and 20.0 (ng).

Results: All samples contained both methamphetamine and amphetamine, while in some samples, especially the white portions of hair, desmethylselegiline could not be detected. The concentrations in pigmented hair were significantly higher than in senile white hair ($p<0.01$ using paired t-test). The mean concentration ratio between pigmented and senile white hair was 3.7 (range 1.8-7.9) for methamphetamine and 2.9 (range 1.8-5.2) for amphetamine. Concentrations ranged from 0.2-3.6 ng/mg for methamphetamine and 0.1-1.4 ng/mg for amphetamine. The mean ratio between amphetamine and methamphetamine was 0.35 (range 0.24-0.60) in pigmented hair with no significant difference from senile white hair ($p>0.10$).

Discussion: The results of this study show that pigmentation plays a role in the incorporation of methamphetamine and amphetamine into human hair. However, because both drugs could be detected in senile

white hair, pigmentation cannot be the only factor involved. Binding to hair proteins might account for a significant part of the drug accumulation in hair suggesting that individuals with low or no pigmentation are still suitable for hair-drug testing programs. The concentration range for both methamphetamine and amphetamine in the Parkinson patients was of the same order as for people abusing methamphetamine although the metabolite/parent drug ratio was higher in the patients receiving Selegiline. Care is needed to avoid misinterpretation of the results as methamphetamine intake, when analyzing forensic samples. Measuring the specific metabolite desmethylselegiline does not solve this problem because not all samples were positive for this substance.

Hair Analysis, Selegiline, Mass Spectrometry

K20 Detection of Ketamine in Nonhuman Primate Hair

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Participants will learn about: 1) principles of extraction and detection of ketamine (KET) in hair using GC-MS, 2) concentrations of KET in nonhuman primate hair after repeated doses of the drug, and 3) detection of KET in hair of young baboons not exposed to the drug but born from mothers treated with KET.

The general anesthetic ketamine (Ketalar®) used in human and veterinary medicine for induction of anesthesia in short surgical procedures and routine veterinary procedures has been identified as a so-called "date-rape" drug for the purpose of "drugging" unsuspected victims and raping them while under the influence of the drug. Its illicit use by teenagers at rave parties has also been reported. The objective of this study was to determine the feasibility of KET detection in hair collected from hair growing primates treated with the drug once a month for longer periods of time. In addition, several hair samples were collected from three young baboons previously not exposed to KET but born from mothers treated with the drug. The aim of this study was: 1) to develop and validate sensitive EI-GC-MS method for the quantitation of KET in hair, and 2) to determine if nonhuman primate hair can be used as an animal model for detection of KET.

Method: Hair was collected from eight stumptail macaques (*Macaca arctoides*), four females and one male living together in a social colony, and two females and one male caged individually. The animals in the social colony received KET twice during the six-month period of time (dose range 7-21 mg per animal). The other three monkeys received 10-13 mg of KET once per month during the twelve-month period. In addition, hair samples were collected from three baboons (*Papio anubis*) less than one year old previously not directly exposed to KET. One hair sample was collected prior to injection of a single dose of KET and others were similarly collected 1, 3, 5, 10, and 20 days after treatment.

Extraction: Hair samples (3-50 mg) were extracted using HCX solid phase extraction columns. Standard curve for KET was prepared by spiking aliquots of negative hair. The LOQ for KET was 20 pg/mg. All standard, control and study samples were spiked with deuterated internal standard, D_5 diazepam and sonicated in methanol for 1 h and incubated in 0.1 N HCl (1ml) over night. A solution of 1.93 M acetic acid (1 ml) and deionized water (10 ml) were added. An analytical column was conditioned with methanol (3 ml) deionized water (3 ml) and 1.93 M acetic acid (1 ml), the sample was added and the column was washed with deionized water (3 ml), 0.1 N HCl (1ml) and methanol (3ml). The final elution from the extraction column was achieved using methylene chloride: isopropanol: ammonia (78:20:2:, v/v/v, 3 ml). All extracts were evaporated to dryness in the stream of nitrogen, dissolved in ethyl acetate (35 µl) and transferred to autosampler vials.

Analytical Procedure: A Hewlett-Packard GC-MS instrument (6890 GC and 5973 MSD) operating in electron ionization (EI) mode was used for the analysis. The column was an HP5-MS. The monitored ions for KET were *m/z* 182 (used for quantitation), 180, and 209, and for D_5 diazepam *m/z* 289.

Results: In general, much lower concentrations of KET in hair were observed in all monkeys from the social colony (737-2,713 pg/mg) than in animals which were receiving KET more frequently (2,869-6,608 pg/mg). In each hair sample collected from young baboons before KET administration, high concentrations of the drug were discovered (2,198-4,500 pg/mg). This strongly suggests *in utero* exposure to KET. No significant differences in KET hair concentrations were observed after a single dose of this drug. KET remained in hair at high concentrations (685-2,435 pg/mg) throughout the 20-day study period.

Date-Rape Drugs, Hair Analysis, Ketamine

K21 Analysis of Drugs From Paired Hair and Urine Samples—Casting a Broader Net of Drug Detection in Pre-Employment Specimens

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During this presentation, the participants will learn about the prevalence of drugs in the hair and urine of job applicants. The participants will also gain an understanding of the different time windows of drug detection in hair and urine and how the pharmacokinetic profile of a particular drug can influence its probability of being detected in urine and/or hair.

Background: The laboratory offers a test for clients in which urine and hair are simultaneously collected from donors for the purpose of drug detection. Urine samples were collected from donors and also hair from the posterior vertex region of the head, as close to the scalp as possible. Hair collected was aligned and the first 3.9cm of hair from the root end, representing an approximate time window of 3 months, was cut and assayed. Drugs detected in urine typically represent the recent past of the potential drug user, within a few days, with the exception of marijuana, which can be present for longer periods in the urine after cessation of use. Drug use in hair represents a much longer time window than urine and a 3-10 day delay may exist before drugs can be detected just above the scalp after entering the hair follicle. Therefore both specimens collected simultaneously, do not reflect the same time window but encompass a broader window than each specimen does alone. By collecting specimens, hair, and urine, the chance of detecting drug should increase. From January 1, 2001 to August 1, 2001, a total of 7,207 paired hair and urine samples were screened for the SAMHSA five (Cocaine, PCP, Amphetamines, Opiates, and Marijuana) drugs of abuse by EMIT for urine and ELISA for hair. Presumptive positives in urine and hair were confirmed by GC/MS or GC/MS/MS.

Experimental: Urine specimens were screened by EMIT utilizing an AU600 Olympus analyzer and Dade Behring reagents for amphetamines, cocaine, barbiturates, marijuana, PCP, opiates, and ethanol. Barbiturate and ethanol results were excluded from the study since they were not assayed in the paired hair sample. If a urine sample screened positive for any of the above drug classes a second aliquot was obtained, extracted by a solid phase extraction or liquid-liquid technique, derivatized (except PCP) and subjected to GC/MS analysis for the appropriate drug(s) and metabolite(s).

Hair specimens aligned in a foil envelope were cut at 3.9 cm from the root end, then further cut into 2-5 mm segments, mixed to ensure homogeneity and 20 mg weighed. The hair was washed with methanol and then placed in hot methanol for 2 hours. The methanol was transferred to a separate tube, evaporated and reconstituted in phosphate buffer. Five separate microtiter plate ELISA assays from International Diagnostic Systems Corp. (IDS) for cocaine, amphetamines, marijuana, opiates, and PCP were used to screen the extracted hair specimens. If positive by ELISA, a second portion of hair from the original 3.9 cm segment was used for confirmation. Cocaine, PCP, amphetamines, and opiates were confirmed by GC/MS and THC and THC-COOH were confirmed by tandem mass spectrometry (GC/MS/MS).

Results: A total of 505 (7.0%) of the 7,207 paired hair and urine samples tested were positive for drugs in both hair and urine, while 723 (10.0%) were positive for drugs in hair only and 329 (4.5%) were positive for drugs in urine only. The total urine positive rate for this population was 11.6% (834), and the total hair positive rate was 17.0% (1,228) with a combined detection rate of 21.6% (1,557). For marijuana, a total of 124 (1.7%) donors were positive in both hair and urine for THC-COOH while 154 (2.1%) were positive in hair only and 93 (1.3%) were positive in urine only. A combined 217 (3.0%) were positive for THC-COOH in urine and 278 (3.8%) were positive in hair. For methamphetamine and/or amphetamine 115 (1.6%) donors were positive in hair and urine and 328 (4.5%) were positive in hair only while only 27 (0.4%) were positive for amphetamines in urine only. Comparing the total urine positive rate to total hair positive rate for amphetamines, 142 (2.0%) donors were positive in urine compared to 443 (6.1%) in hair. For cocaine the most significant difference was seen comparing hair and urine positives. A total of 52 (0.7%) of donors tested positive for cocaine in both hair and urine and only 5 (0.07%) tested positive in urine only while 273 (3.8%) were positive for cocaine in hair only. The overall detection rate in hair for cocaine was 4.5% (325) compared to 0.8% (57) in urine specimens from the same population.

This study shows that the analysis of hair increases the number of specimens which are positive for drugs of abuse, when compared to urinalysis using laboratory established cut-offs for both hair and urine. Furthermore, the combination of both urine and hair provides the greatest chance of detecting drug use. The most dramatic differences were seen with cocaine and methamphetamine positive rates, while the incident of marijuana positives in hair versus urine was considerably less marked. One possible explanation is the pharmacokinetic differences between these drugs. Drugs which have relatively short half-lives (i.e., cocaine) are more likely to go undetected in the urine of a drug user while hair provides a longer history of drug use. Another explanation may be due to the pattern of drug use or the abuse liability of a particular compound. Drugs used in a more regular or chronic fashion would be more readily detected in hair due to accumulation of drug than drug that is used rarely or occasionally.

Paired Hair and Urine, Prevalence, Drug Testing

K22 Relationship Between In Utero Methadone Exposure and Neonatal Outcomes

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The objective is to present information on the relationship between maternal methadone dose and neonatal outcomes in an agonist therapy program for opiate dependent pregnant women.

In utero drug exposure has been associated with a host of negative maternal and fetal outcomes. Currently, the only recommended pharmacotherapy for opiate dependence in pregnant women is methadone, a full μ agonist. It is still a matter of controversy whether maternal methadone dose is correlated to the severity and duration of neonatal abstinence syndrome (NAS). This syndrome is characterized by increased startle reflex, tremors, inability to self-quiet, poor feeding, abnormal sleep patterns, diarrhea, fever, and seizures in the infant. Depending on the severity of NAS, pharmacotherapy intervention may be required. Unlike other studies, this research protocol has documented illicit drug exposure and methadone dosage over an extended gestational period. This study evaluates in utero methadone exposure in pregnant heroin abusers participating in an agonist therapy program and the effects of this treatment on neonatal outcomes. The authors hypothesized that maternal methadone dose would not correlate to birth weight, length, and head circumference, but could influence the severity and duration of NAS.

Eighteen heroin dependent pregnant women were enrolled in an intensive methadone and behavioral modification treatment program at the Center for Addiction and Pregnancy (CAP), between 8 to 28 weeks of gestation and followed throughout their pregnancy. Each participant provided informed consent and submitted to tri-weekly observed urine specimens which were analyzed for cocaine and opiates by EMIT (Dade Behring, San Jose, CA). The mean number of weeks on the study was 20.8 $\pm$ 6.2 (SD). Mean fetal age at delivery was 37.9 $\pm$ 4.0 (normal gestation 40 weeks) with a range from 26 to 42 weeks. The mean daily methadone dose was 76.4 $\pm$ 14.4 mg with a range of 45 – 100 mg/day. Of the 18 women, 44% were positive for hepatitis C, 11% for hepatitis B and 6% for HIV. All women had a history of cocaine and opiate abuse, 78% used nicotine and 17% self-reported using alcohol while pregnant. 39% of the women self-administered cocaine and opiates during gestation as documented by urine test results. Illicit opiates were self-administered by 11% of the women. 22.2% had positive toxicology tests at delivery for opiates, cocaine and/or barbiturates. The mean NAS peak score was 8.8 $\pm$ 4.9 (range 3-19) and the mean duration of withdrawal was 8.6 $\pm$ 6.7 days (range 3-24). Mean birth weight, length, and head circumference were 2889.2 $\pm$ 911.2 g, 46.6 $\pm$ 7.2 cm, 32.8 $\pm$ 1.9 cm, respectively. There were no significant differences in birth weight, length, and head circumference between methadone exposed and reported normal values for non-exposed babies (Britton, J. Reproductive Medicine, 1993;38:215). No significant correlation was seen between methadone dose and NAS peak or duration. However, there was a correlation between NAS peak score and the duration of NAS symptoms ($r = 0.94$, $p < 0.001$). In this preliminary report of 18 methadone maintained pregnant women, followed for the majority of their gestation, methadone dose did not significantly affect neonatal outcome measures, including physiological parameters, and the severity and duration of NAS.

Methadone, In Utero Opiates, Neonatal Abstinence Syndrome

K23 Stability of Many Prevalent Drugs Found in Postmortem Bloods From Medical Examiner Cases

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This study was designed to measure the stability of certain drugs of abuse in postmortem blood specimens taken from previous medical examiner cases. The objectives were to: 1) examine the preservation of drugs over a ten-year period to measure the rate of change in drug concentration as related to drug stability and whether the stability increases or decreases at a greater rate after five years; and 2) find the optimum storage

time for specimens before the validity of the drug concentration is questioned. As a result of this study, the findings showed that all but two of the twenty-one drugs examined exhibit a good stability in concentration within the initial two years, prior to showing decreases in drug stability. Due to these findings, the optimum storage time for postmortem blood specimens stored under similar conditions should not exceed two years.

Analytical Methods: The drug analysis for this study was performed using the same instrumentation and methods as the initial analysis of the blood specimens, specifically the 5880 Hewlett Packard (HP) gas chromatograph, equipped with dual nitrogen phosphorous detectors, two fused silica columns (DB-5, 15m, 0.25mm i.d., film thickness 0.25um; and a DB-17, 15m, 0.25mm i.d., film thickness 0.15um). Two HP 5880A integrators were used to monitor the signals and an HP 7673A autosampler was used to automate the runs. For the volatile analysis of ethanol, a Perkin-Elmer HS-40 autosampler injected the headspace into a Perkin-Elmer Autosystem gas chromatograph equipped with a 30m x 0.32mm, 1.8um film Restec BAC1 column.

The drugs analyzed were: Benzodiazepines (Diazepam, Nordiazepam, Chlordiazepoxide and Nordiazepam), Cocaine, Opiates (Codeine, Hydrocodone and Oxycodone), Tricyclics Antidepressents (Amitriptyline, Nortriptyline and Imipramine), Doxepin, Nordoxepin, Diphenhydramine, Fluoxetine, Thioridazine, Methadone, Verapamil, Propoxyphene, Norpropoxyphene, Meperidine, and Ethanol. The postmortem blood specimens were stored at 6°C in 20ml glass tubes containing 55mg of sodium fluoride preservative and sealed with rubber stoppers.

Results: Diazepam showed good stability for the first three years with decreases in drug concentration ranging from 7-19%, but poor stability in subsequent years with an average decrease of 33%. Decreases of 44% and 57% were noted at the fourth and fifth year, respectively. Similarly, nordiazepam showed variable stability for the first three years, followed by poor stability for the remaining seven years. Chlordiazepoxide is unstable in stored or decomposed biological specimens; it tends to form in vitro desoxychlordiazepoxide, which further degrades to nordiazepam. Chlordiazepoxide in this study shows poor stability, whereas nordiazepam showed a variable decrease in concentration ranging from 0.2% at year seven to 5% at year ten. Cocaine showed decreases in concentrations of 94% in year one to 98% in year four. This phenomenon is concentration-dependent, lower levels of cocaine were not detected even after one year before reanalysis. These findings are consistent with previous studies [1-3] examining the stability in blood of 1.0 mg/L cocaine concentration at 16°C, where a 30% loss occurred after 36 days, and a 7% loss after one day. Codeine showed good stability over the entire course of the study. Hydrocodone showed good stability for the first two years of the study, while a significant decrease of 48% concentration was noted in the eighth year of the study. Oxycodone revealed a variable decrease in concentration over the first eight years reaching a 58% decrease at the ninth year. Amitriptyline demonstrates a variable decrease in concentration over the years, ranging from 17 to 50%. Nortriptyline has a decrease in concentration of 75 to 78% between years five and seven, respectively. Imipramine shows a variable decrease in concentration for all of the years. Doxepin showed a variable decrease in concentration, ranging from 7 to 74% in the ten-year period. Nordoxepin showed decreases in concentrations as high as 73% in the ninth year and 80% in the tenth year. Diphenhydramine is a very stable drug, averaging a decrease of only 15% from one to ten years. Fluoxetine has a good stability for the first two years of the study and variable stability thereafter. Thioridazine shows good stability over the course of the study, with an average decrease in concentration of 11% for the first four years, increasing to 45% by the tenth year. Methadone shows good stability throughout the study. Verapamil reaches the highest decrease in concentration of 42% at year ten, averaging a decrease of 17 to 39% during the previous years. Propoxyphene is more stable for the first six years of the study and shows a decrease in concentration of 34% for the tenth year. Norpropoxyphene exhibits good stability for the first four years and a decrease in concentration of 60% for the eighth year. Meperidine averages only a 15% decrease in concentration from year one to year ten.

The differences detected in the ethanol analyses were not statistically significant by the student t-test except for the seven year-old specimens ($p=0.04$).

Drug Stability, Gas Chromatography, Postmortem Blood Specimens

K24 Postmortem Tissue Distribution of Olanzapine and Citalopram in an Overdose

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The objective is to determine the tissue distribution of olanzapine and citalopram in an overdose fatality and to contribute to the scientific literature pertaining to the therapeutic and toxic concentration of these drugs.

Olanzapine is a thienobenzodiazepine derivative used as an antipsychotic for the treatment of schizophrenia in the U.S. since 1996. It interacts with dopamine-d_2 and serotonin 5-HT 2A receptors, and antagonizes dopamine-d_1, d_4 and serotonin 5-HT 2C, 5-HT3, α-1-adrenergic, H1 histaminic and muscarinic receptors. Citalopram is among the newest orally administered selective serotonin reuptake inhibitor antidepressants currently prescribed. Its chemical structure is unlike that of other SSRIs, tricyclic, tetracyclic, or other antidepressants. The mechanism of action of citalopram as an antidepressant is presumed to be linked to the potentiation of serotonin in the central nervous system resulting from its inhibition of CNS neuronal reuptake of serotonin. Olanzapine is dispensed as 2.5, 5, 7.5, and 10 mg tablets for oral administration. The recommended starting daily dose is 5-10 mg in adults, with peak plasma concentrations averaging 5-10 ng/mL after approximately six hours. Citalopram is available as a racemate as 20 and 40 mg tablets. Oral doses of 30-60 mg per day result in serum concentrations of 9-200 ng/mL. To date, little scientific literature exists about the therapeutic concentrations or postmortem tissue distributions of these drugs.

The case of a 40-year-old Caucasian male with a medical diagnosis of paranoid schizophrenia found dead in a group home is presented. No anatomic cause of death was determined at autopsy and multiple biological samples including heart blood, femoral blood, urine, vitreous humor, cerebrospinal fluid, brain, liver, kidney, spleen, lung, bone, heart, and gastric contents were collected. The heart blood and urine were subjected to routine comprehensive toxicological analysis. This testing included volatile analysis by headspace gas chromatography; enzyme immunoassay screening of the urine for amphetamines, benzodiazepines, cannabinoids, cocaine metabolites, opiates, and phencyclidine; acidic/neutral, benzodiazepine, and basic drug screening by GC; opiate screening by modified immunoassay; and acetaminophen, salicylate, and ethchlorvynol screening by colorimetry. Electrolyte analysis was performed on the vitreous humor. Olanzapine and citalopram were the only drugs detected through routine analysis, both at overdose concentrations.

Quantitation of olanzapine and citalopram in biological specimens was performed by GC-NPD. Specimens were diluted when necessary so that the concentrations were within the calibration curve. Liver, lung, brain, spleen, kidney, and heart tissues were homogenized with deionized water (6g + 30 mL deionized water) in a commercial blender. Bone was prepared by soaking whole pieces (1 g) and slivered pieces (1 g total mass) overnight in aliquots of both water and methanol. A basic liquid-liquid extraction with promazine as internal standard was performed, followed by back extraction with sulfuric acid. The acid phase was buffered to pH 9.2 with Tris buffer, re-extracted in hexane:isopropanol (9:1) solvent, evaporated to dryness, and reconstituted in methanol. A stock standard

reference solution of each olanzapine and citalopram (100 mg/L) was prepared in methanol. Calibrators were prepared by spiking drug-free blood with the stock methanolic solution to give concentrations of 0.5, 1.0, 1.5, and 2.0 mg/L for citalopram and 0.1, 0.3, 0.5, 0.8, and 1.0 mg/L for olanzapine. A positive control was prepared at 0.6 mg/L. Olanzapine and citalopram concentrations in the control and specimens were calculated from linear regression of the calibrator responses based on peak-area ratio. The presence of olanzapine and citalopram was confirmed by a combination of GC retention time and full scan electron impact mass spectrometry. The table below presents the drug quantitations for each tissue. As can be seen from the table, citalopram and olanzapine were readily detected in both biological fluids and tissues.

	Drug Concentrations- mg/L or mg/kg	
Specimen	Citalopram concentration	Olanzapine concentration
Heart blood	3.35	1.38
Femoral blood	1.65	1.11
Urine	32.43	60.24
Vitreous humor	0.84	4.47
CSF	0.33	Not detected
Spleen	11.12	2.78
Liver	10.71	6.47
Brain	7.41	2.13
Kidney	7.00	2.39
Heart	4.59	1.72
Lung	49.16	38.36
Gastric contents	56.48	4.47
Bone	3.56	Not detected

The heart blood concentrations of olanzapine and citalopram were several times the reported therapeutic levels. Therefore, the cause of death was ruled acute intoxication by the combined effects of citalopram and olanzapine, and the manner was ruled accidental.

Citalopram, Olanzapine, Postmortem Forensic Toxicology

K25 Distribution of Quetiapine in Postmortem Specimens

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After attending this presentation, the attendee will: (1) understand the pharmacological action of Quetiapine, (2) be able to describe a simple assay for its detection in biological matrices, and (3) possess information regarding drug concentrations in postmortem specimens.

Quetiapine is a second generation antipsychotic medication approved for use in the U.S. in 1997. Preclinical studies have suggested that this dibenzothiazepine is an atypical antipsychotic with many similarities to clozapine. It is structurally related to clozapine and olanzapine. The recommended dose for clinical efficacy is 300-450 mg/day administered in two doses. The pharmacological effect is primarily due to antagonistic binding to serotonergic (5HT2) and dopaminergic (D2) receptors. After oral administration, peak plasma concentrations are achieved at 1.5 h with an elimination half life of 6 h. A single oral dose of 75 and 450 mg produced peak serum quetiapine concentrations of 0.14-0.37 mg/L and 0.19-0.63 mg/L, respectively. Steady state plasma concentrations are achieved within two days after the start of dosing. Quetiapine is extensively metabolized, primarily by sulfoxidation and oxidation, to inactive metabolites. The most common clinical effects reported in overdose were hypotension, tachycardia, prolonged QTc, and somnolence due to α-adrenergic and histaminic receptor blockade.

Since this drug has only recently been released on the U.S. market, there is limited published data regarding therapeutic, toxic, and lethal concentrations. Therefore, determination of quetiapine in 4 postmortem cases is reported:

Case #1 A 27-year-old black male found dead at home on the bathroom floor.
Case #2 An 80-year-old white female found dead in bed at a nursing home.
Case #3 A 71-year-old white male found dead at home on the kitchen floor.
Case #4 A 39-year-old black female admitted to hospital after being found unresponsive at home.

Blood and urine specimens were subjected to comprehensive toxicological testing which included volatiles by headspace gas chromatography; acetaminophen, salicylate, and ethchlorvynol screening by colorimetry; acidic/neutral and basic drug screening by liquid-liquid extraction followed by GC-FID or GC-NPD; benzodiazepine screening by GC-ECD; modified opiate immunoassay screening of blood; and immunoassay for amphetamine; cocaine metabolite, cannabinoids, phencyclidine, benzodiazepines, and opiates in urine.

Quetiapine was identified by capillary column (RTx-50) gas chromatography with nitrogen phosphorus detection (Hewlett-Packard [HP] 5890 or 6890) after basic liquid-liquid extraction. Promazine was utilized as the internal standard. Quantitation was achieved by assaying a single point calibrator at 1 mg/L concurrently with case specimens. Specimens were diluted when necessary to produce a relative area similar to the calibrator. Negative and positive (0.5 mg/L) matrix matched control samples were assayed with each run. Confirmation was achieved by full scan electron impact gas chromatography/mass spectrometry using an HP 5973 MSD with a DB-5 capillary column. For cases #1 and #4, tissues were also assayed for quetiapine using a three point calibration curve (0.50, 1.0, and 2.0 mg/L) with a correlation coefficient typically >0.99.

Quetiapine had a relative retention time of 1.59 minute on the RTx-50 column, eluting after the I.S. and after thioridazine but before trazodone. By GC/MS, the base peak of quetiapine was *m/z* 210, with a molecular ion at *m/z* 383 and other prominent ions at *m/z* 144, 239 and 321.

The table below illustrates the quetiapine and other drug concentrations (mg/L) determined in the biological matrices from four postmortem cases with the cause and manner of death:

Case	Heart Blood	Femoral Blood	Other Drugs (ht bld)	Cause	Manner
#1	0.72	0.28	Haloperidol 0.02	Acute Intoxication by quetiapine	Accidental
#2	0.51	-	ND	Atherosclerosis Ankle Fracture	Accidental
#3	0.44	-	Diazepam 0.27 Nordiazepam 0.13 Oxycodone 0.66 Hydroxyzine 0.38 Venlafaxine 2.08 DMVenlafaxine 1.98	Hypertensive ASCVD	Natural
#4	11.20	7.76	Ibuprofen 29.4 Verapamil 1.72 (f)	Acute Intoxication by combined effects of IBU/QT/TCA/V	Suicide

ND= Not detected; -=Specimen not collected; ht bld= heart blood; f= femoral blood.

The heart blood concentrations of quetiapine measured in cases #2 and #3 were within the established therapeutic range for patients prescribed high daily doses of quetiapine. For case #1, the heart blood quetiapine concentration was only slightly elevated compared with published therapeutic levels. However, there were no significant findings at autopsy and empty pill vials were found beside the deceased. This death suggests that quetiapine may exhibit a low therapeutic index. In case #4, the deceased survived for 20 hours in the hospital. Hospital urine drug testing was positive for tricyclic antidepressants. The quetiapine concentration in a hospital serum specimen collected on admission was 5.36 mg/L.

Forensic Toxicology, Antipsychotic, Quetiapine

K26 The Distribution of Gamma-Hydroxybutyric Acid (GHB) in Both Ante and Postmortem Specimens, of a Single Fatality After Long-Term Storage

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The objective is to document GHB concentrations in various specimens collected from a single GHB fatality at four different times of specimen collection and after long-term storage.

Content: A 15-year-old female victim allegedly consumed an unknown amount of a drink laced with a mixture of GHB/GBL on 1/16/99 at approximately 11:30 p.m. She became violently ill and her friends decided to take her to the hospital when she stopped breathing, arriving on 1/17/99 at approximately 5:00 a.m. In the course of her treatment, several specimens were collected from her at three different times. Upon admission to the hospital, five tubes of blood with the following color tops were collected: red, yellow, green, blue, and purple. Also an admission urine specimen was collected. At 11:00 a.m. a spinal fluid specimen was collected and between 12:35 and 12:55 p.m., four more tubes of blood were collected with the following color tops: yellow, green, purple, and gray. At 7:34 p.m. on 1/17/99 she was pronounced dead, having never regained consciousness. The cause of death was GHB intoxication with no other drugs present including ethanol. At autopsy the next day (1/18/99) the following specimens were collected: urine, heart blood, femoral blood, vitreous fluid and bile. All of the specimens were analyzed either soon after the autopsy or after long-term freezer storage. The original specimens analyzed were the 5:00 a.m. red top blood, the hospital urine, the postmortem heart blood, and the postmortem urine. These specimens were sent out to a reference laboratory for analysis on 1/19/99. The remaining specimens were stored in the refrigerator until March 6, 1999, at which time they were moved to the freezer where they remained until July of 2001. At this time the specimens were analyzed in duplicate at the Wayne County Medical Examiner's Office using a modified version of the United Chemical Technologies GHB solid phase extraction method which derivatizes GHB with no conversion to GBL. This method directly measures the GHB di-TMS derivative by EI GC/MS with no conversion to GBL and utilizes d-6 GHB as the internal standard with a specimen size of 200 microliters. The method has a linearity range from 2.5 to 100 mcg/mL, and a recovery between 35% and 60% (specimen type dependent). Variation in concentration between analytical runs of both internal and external controls and specimens was no greater than 15%, n=54. Table 1 shows the results of analysis of the antemortem specimens and Table 2 shows the results for the postmortem specimens.

Table One - Antemortem

Specimen	GHB in mcg/ml original analysis	re-analysis
Red Top Bld 5:00a.m.	510	466
Yellow Top Bld 5:00a.m.	NP	566
Yellow Top Bld 12:55p.m.	NP	187
Green Top Bld 5:00a.m.	NP	QNS
Green Top Bld 12:35p.m.	NP	232
Purple Top Bld 5:00a.m.	NP	QNS
Purple Top Bld 12:55p.m.	NP	242
Blue Top Bld 5:00a.m.	NP	418
Gray Top Bld 12:55p.m.	NP	93
Spinal Fluid 11:00a.m.	NP	220
Urine 5:00m	2300	QNS

NP = test not performed
QNS = quantity not sufficient for analysis

Table Two – Postmortem

Specimen	GHB in mcg/ml Original analysis	Re-analysis	Endogenous GHB in postmortem specimens*
Heart Blood (NaFl)	15	12	1.6 – 36 Average = 12
Femoral Blood	NP	18	1.7 – 48 Average = 11
Urine	150	102	0 – 14 Average = 4.6
Urine at pH = 14 for 30 min.	NP	167	NA
Bile	NP	48	Unknown
Vitreous Humor	NP	127	Less than 10 mcg/ml

*Anderson, D.T., Kuwahara, T., "Endogenous Gamma Hydroxybutyrate (GHB) levels in Postmortem Specimens", Abstract CAT/NWAFS/SWAFS/SAT combined meeting, Las Vegas, Nevada, Nov. 7, 1997.

The concentrations of GHB at the various collection times would indicate a reduced clearance of GHB in a patient who is alive but severely compromised by respiratory and metabolic acidosis (admission blood pH=6.8) from the respiratory depression caused by the high dose of GHB. The lower GHB concentration in the 12:55 p.m. gray top tube blood as compared to the yellow, green, and purple top tubes collected at around the same time cannot be explained at this time. The *apparent* half-life of GHB calculated in narcoleptic patients is 53 +/- 19 minutes for two 3gram doses (Scharf et al., 1998) but this is dose dependent. Even after 14 hours there is still evidence of past GHB ingestion in the postmortem urine and vitreous fluid and perhaps even the bile. More bile specimens should be analyzed to determine a postmortem GHB range in this specimen type. Table 3 has vitreous humor results from two other GHB fatality cases, both of these cases involved deaths with less survival time and no hospitalization with both victims being found dead some 5 to 7 hours after they were last seen alive. Also it is apparent that GHB conversion to the lactone does occur over time especially in a postmortem urine with a low pH such as in this case. The conversion back to GHB was accomplished because any GBL in the urine should have been from GHB excretion. Ingested GBL is rapidly converted enzymatically in the blood and liver to GHB by a lactonase enzyme. The converted GBL is then excreted as GHB. However, due to the pH dependent chemical equilibrium that exists between GHB and GBL, the low pH (4.2) of the postmortem urine would facilitate a shift in the equilibrium over time such that some of the GHB is converted to its lactone GBL. GBL analysis was not performed on any of these specimens.

Table 3 – Two Additional GHB Fatality Cases

Postmortem Specimen Case 1	GHB in mcg/mL	Ethanol in g/dL
Heart Blood	66	ND
Femoral Blood	77	ND
Vitreous Humor	85	ND
Urine	1260	ND
Postmortem Specimen Case 2		
Blood	400	.22
Vitreous Humor	212	.12

Specimen Storage, GHB Distribution, Ante and Postmortem Specimens

K27 Zolpidem Intoxication

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The participant will learn the tissue distribution of zolpidem in three cases.

Zolpidem (Ambien®) is a hypnotic agent. Case histories and toxicological findings from three zolpidem intoxication cases are

presented. In the first case, a 31-year-old white female was found unresponsive in her bed. A suicide note and two empty medication bottles labeled as Ambien® and Benadryl were found at the scene. In the second case, a 49-year-old white male was hospitalized due to an overdose and died nearly two days later. In the third case, a 35-year-old female was found unresponsive in her bed. Ambien® and other medication bottles were found at the scene. She had a previous suicide attempt.

Zolpidem was detected in postmortem specimens using screening procedure for basic drugs. Two milliliters (2 mL) of blood were extracted with 10 mL of hexane-ethyl acetate (1:1) at pH 10 using 100 µL of 20 mg/L trifluoperazine as am internal standard. After back extraction with 2 mL of 1N sulfuric acid, and re-extraction with 6 mL of hexane-ethyl acetate (1:1) at pH 10, the residue was reconstituted with 50 µL of ethyl acetate and analyzed by dual column GC/NPD. One 10m x 0.53mm HP-5 column and one 10m x 0.53mm HP-17 column were used. The retention times of zolpidem and the internal standard, trifluoperazine, on the HP-5 column were 8.23 min and 7.37 min, respectively. The retention times of zolpidem and the internal standard, trifluoperazine, on the HP-17 column were 12.4 min and 7.81 min, respectively. Zolpidem was quantified by GC/NPD. The presence of zolpidem was confirmed by full spectrum GC/MS. The base peak and the molecular ion peak of zolpidem were *m/z* 235 and *m/z* 307, respectively. Some of the other characteristic ions were *m/z* 72, *m/z* 92, *m/z* 115, and *m/z* 219.

The tissue distribution of zolpidem for the first case was as follows: blood 44.5 mg/L and liver 81.2 mg/kg. The first case also had the following in blood: diphenhydramine 34.6 mg/L and ethanol 83 mg/dL. The cause of death for the first case was zolpidem and diphenhydramine intoxication. Suicide was listed as the manner of death.

The tissue distribution of zolpidem for the second case was as follows: pleural fluid 0.13 mg/L and liver 0.27 mg/kg. The second case also had the following in pleural fluid: verapamil 6.18 mg/L, propanolol 1.03 mg/L, amitryptiline 0.53 mg/L, meprobamate 3.83 mg/L papaverine 0.10 mg/L and nordiazepam 0.17 mg/L. The cause of death for the second case was multiple drug intoxication, and the manner of death was suicide.

The tissue distribution of zolpidem for the third case was as follows: blood 0.39 mg/L and liver 1.49 mg/kg. The third case also had the following in blood: temazepam 1.20 mg/L and sertraline 0.25 mg/L. The cause of death for the third case was sertraline, temazepam, and zolpidem intoxication, and the manner of death was suicide.

According to Baselt, two adults who die following acute overdose with zolpidem and ethanol had postmortem blood concentrations of 0.8-0.9 mg/L for zolpidem and 240-250 mg/dL for ethanol (Khodasevitch; Deveaux et al.). Two women who died after acute overdosage with zolpidem as a sole agent had postmortem heart blood concentrations of 4.3 mg/L and 7.9 mg/L (Baselt; Lichtenwalner and Tully). The first case had a postmortem blood concentration of 44.5 mg/L of zolpidem.

Zolpidem, Toxicology, Ambien

K28 Medications, Strange Intoxications, and Violent Crime: Psychiatric, Toxicology, and Legal Considerations

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The proliferation of newer psychotropic drugs has been accompanied by criminal defenses attributing blame to these medicines for violent and other crimes. These cases were popularized by early suggestions that the antidepressant Prozac might increase the likelihood of suicide. Criminal and civil complaints invoking medicine-induced crime inspired consideration of other psychotropic and other prescriptions that might also be raised as contributing factors to crime. The lecture begins with a forensic psychiatrist-psycho-pharmacologist's review of cases in which drugs have been implicated, successes and failures.

This program explores the role medicines – psychotropic and others – may play in violent behavior. The presenting psychiatrist reviews potentials of single medicines, drug interactions, and idiosyncratic intoxication, separating myths and facts based on case reports and available literature. The problem of drug reporting is also raised.

Violent crimes have also been reported in individuals who were using newer man-made drugs, such as flunitrazepam, crystal methamphetamine, and gamma hydroxybutyrate. In that regard, the courts' sophistication about the influence of designer drugs has lagged published reports about their effects. The presenting toxicologist review case reports linking intoxication with these newer agents with violence, and the nature of the associated behavior.

Next, a toxicologist lab director presents the program the relationship, metabolically, of legal and illicit drugs to violent crime. This includes research findings from the literature about when individuals under the influence would be expected to erupt in violence, and special considerations at the level of the laboratory for assessing the drug-violence link.

Detection of drugs and medicines, and their relationship to the instant offense, also challenges the forensic scientist. Techniques and standards for the laboratory measurement and analysis of suspected drug or medicine influenced cases is reviewed.

Finally, the forensic psychiatrist presenter offers guidelines for forensic psychiatrists who are charged with assessing possible medication-related criminal cases. In addition, strategies for approaching and assessing such cases – from the both the defense and prosecuting attorney's perspective – are presented.

A legal perspective is also included in the program; a prosecutor discusses the dilemmas of charging offenses that may be drug mediated in a climate of alternatives to incarceration and prison overcrowding. Participants gain insight into decision-making that distinguishes how such cases are handled and disposed of in such serious cases, such that they better recognize the history and findings to which a prosecutor of judge might be especially sensitive or responsive.

Toxicology, Forensic Psychiatry, Criminal Law

K29 Fentanyl Related Deaths on the Rise in North Carolina

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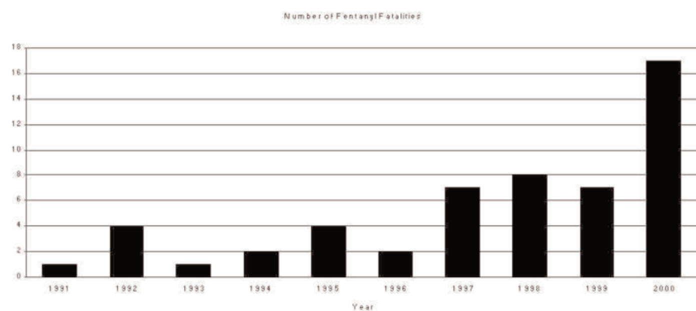
After observing this presentation, the participant should have an understanding of the increased incidence of fentanyl related deaths, especially in the State of North Carolina.

Fentanyl is a synthetic narcotic analgesic that has dramatically increased in medical use since 1990. The increase in medical use, in part, is directly related to the fact that in 1990, fentanyl was released in a transdermal patch form, Duragesic®. A recent report documented that the increase in medical use contrasted to a decrease in abuse during the six-year period from 1990. At the North Carolina Office of Chief Medical Examiner (NC-OCME), an increase in the number of fentanyl positive results the last few years was noted. Therefore, all fentanyl positive results in the State of North Carolina from 1991 to 2000 were retrospectively reviewed to determine if there was an increase in the number of fentanyl-related deaths.

Methods of analysis: Testing for fentanyl is not an automatic screen in the authors' laboratory. Instead, assignment of a fentanyl screen is determined by case history and autopsy findings or may later be assigned

if there is no anatomic cause of death and routine toxicology is negative. Specimens were screened utilizing either RIA (1990-1999) or ELISA (1999-present) with a 1 ng/mL cutoff. Prior to changing immunoassay methodology, a parallel validation study analyzing actual specimens was performed to compare sensitivities and specificity. Quantification and confirmation analyses were performed by first isolating fentanyl by basic extraction of a 1-5 mL (g) specimen. Underivatized specimens were analyzed by gas chromatography/mass spectrometry (GC/MS) utilizing selective ion monitoring and a four-point calibration curve with a linear range of 0.005 to 0.10 mg/L. Quantification of fentanyl (245, 146, and 189) was based on the ion ratios of integrated ion peak-areas compared to one of two internal standards, fentanyl-d5 (250, 151, 194) or codeine (299, 229, 162).

Study design and results: A total of 71 fentanyl positive deaths were identified by NC-OCME for the 10-year period. Eighteen of the cases were rejected on the bases of non-drug related causes of death. The non-drug related causes of death include homicides, surgical, and natural deaths with therapeutic fentanyl levels. The average number of medical examiner cases per year during the study period was 8609, standard deviation of 206. The number of fentanyl related deaths averaged two cases per year for the first six years of the study (see figure). During this period, the fentanyl blood levels ranged from 0.007 to 0.044 mg/L. From 1997 to 1999, the average number of cases increased to seven per year, a three-fold increase over the previous six years. During this period, the fentanyl blood levels ranged from 0.002 to 0.055 mg/L. In 2000, the number of fentanyl-related deaths increased to 17 cases. This represents a two-fold increase over the previous three years and an eight-fold increase over the first six years of the study. In 2000, the fentanyl blood levels ranged from 0.004 to 0.077 mg/L, which included the highest fentanyl level reported in the study. Liver fentanyl levels during the study period ranged from 0.01 to 0.31 mg/kg. Of the 53 accepted fentanyl positive deaths, 27 of the cases had additional drugs detected on toxicological evaluation. The fentanyl blood level in these 27 cases ranged from 0.002 to 0.022 mg/L. It should be noted that in only 10 of the 27 cases were the additional drugs determined to be of significance by the medical examiner and/or forensic pathologist. In these 10 cases, the fentanyl blood level ranged from 0.008 to 0.027 mg/L. The most common manner of death in the reviewed cases was accidental. White males were the most common decedent with whites representing 92% of the cases and males representing 64% of the cases. Two case studies will be presented: a case of a 44-year-old white male who died from the abuse of Duragesic® patches and a case of a 27-year-old white male who died from the accidental misuse of a prescribed Duragesic® patch.



Conclusions: This study indicates that fentanyl use and subsequent fentanyl-related deaths in the State of North Carolina has dramatically increased over a four-year period since 1996. In contrast with previous reports, the data indicate that while medical use of Duragesic® patches increased, fentanyl related deaths from abuse/misuse either remained stable or increased over the ten-year time period of our study, instead of declining.

Fentanyl, Duragesic®, Medical Use

K30 Driving Under the Influence of Methadone—Experiences From Berne, Switzerland

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Practical cases and an overview showing the problems associated with driving under the influence (DUI) of methadone, focusing on drivers treated in a medical substitution program, are presented. Methadone alone is not regarded as impairing coordination and alertness sufficiently to compromise driving. However, the data show multiple drug abuse in these drivers, 35% of those showing such patterns of abuse being involved in severe road accidents. Since multiple drug abuse in some methadone recipients is clearly occurring, is compromising driving, and is a public health risk, this must be addressed in any new law on DUI.

Current laws on DUI are inconsistent. Consistency and improvement are recommended. An analysis of practical cases from the view of a forensic toxicologist is necessary for gathering data that can serve as a practical basis for the regulatory decisions of federal authorities. Furthermore, these results may serve as an additional element in preparing practically applicable future law for the protection of driver and traffic safety.

Currently, based on a "Methadone Report" of the Swiss National Bureau of Public Health 1995, driving under the influence of methadone is regarded as not affecting the capability to drive a car, if certain conditions are fulfilled. These are: 1) methadone must be prescribed for at least six months by a physician and the personality of the patient must be judged as stabilized, 2) methadone is administered orally on a daily basis (maximum dose 120 mg/day), 3) the consumption of alcohol and other psychotropic substances is "forbidden," and 4) the assumption is that all methadone recipients consume no other psychotropics. The data show that this is not the case.

Driving under the influence of alcohol results in withdrawal of the driver's license for a period of several weeks to several months. In cases of chronic alcoholism and repeated offenses the driver's license is withdrawn permanently, until abstinence from alcohol is proven and a psychiatric evaluation and psychological tests indicate full recovery and driving capability. However, this is currently not the case in cases associated with driving under the influence (DUI) of methadone. There is no obligation to report a person on methadone substitution therapy to the legal or administrative authorities due to medical confidentiality. Furthermore, there is no obligation to report the additional use or misuse of drugs such as neuroleptics, antiepileptics or licit or illicit psychotropic substances, even if such use or misuse is recognized by the physician. Currently, driving under the influence of methadone and additional prescribed medicines or illicit drugs usually results in a rather short withdrawal of the driver's license. An existing law (drug-dependent persons are not allowed to drive) is often not applied to persons driving under the influence of methadone due to the lack of facts and experience with problems associated with such driving.

A population of 941,000 were analyzed retrospectively. The eligible drivers (age group 20 – 64) consisted of 580,000 people, from which 2,042 were currently known to be in a methadone substitution program (paid by public health agencies or medical insurances). From these, an unknown percentage are permanently driving.

The number of the cases of "driving under the influence of alcohol" resulting in withdrawal of the driver's license is rather constant in the last years (1997-2000: 2,200 cases/year). Also the known number of DUI on methadone remained rather constant (1997-2000: 45 cases/year). However, the situation of DUI of alcohol in combination with cannabis or DUI of cannabis alone changed dramatically in the last years due to the availability of cannabis and its widely tolerated consumption. Typically, urine tests, which are positive for only one drug, reveal THC-metabolites.

Importantly, in contrast, urine tests of DUI of methadone cases revealed the abuse of a variety of prescribed and/or illicit drugs, also in

combination with alcohol. From 1997-2000, 166 DUI cases of patients in a methadone substitution program, who underwent a routine police control or were stopped by the police after a violation of traffic rules or a traffic accident were retrospectively analyzed. Only 6 of 166 patients revealed methadone as the only detectable psychotropic substance. Therefore 160 patients in a methadone substitution program revealed the abuse of multiple substances, sometimes in combination with alcohol. Of these, 35% resulted in severe traffic accidents. This clearly demonstrates that multiple drug ingestion is occurring in patients on methadone substitution programs and this is contributing to driving risk.

The authors support the authorities in protecting those individuals in these programs who genuinely wish to abstain from drugs and ingest only methadone, whilst penalising polydrug abusers who drive and pose a risk to themselves and others. The authors strongly recommend the application of systematic, random screening tests for patients in substitution programs, to detect multiple drug use, enforce the obligation to report such patients to administrative or federal authorities due to the potential hazard for other people, and to create regulations or laws on a federal level to withdraw the driver's license permanently from these polydrug abusers until drug abstinence, recovery, and full driving capability has been proven. Hair analysis has been used effectively in other countries.

Methadone, Drugs and Driving, Traffic Safety

K31 New Studies on the Pharmacokinetics of Ethanol

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Those attending this presentation will learn about the absorption, distribution, metabolism and excretion, of man's favorite drug - ethyl alcohol. Results of new experiments on the disposition kinetics of ethanol in arterial and venous blood will be presented as well as studies on absorption kinetics of ethanol in patients operated on for gastric bypass. These topics have importance in forensic science because impairment of a person's performance and behavior after drinking alcohol is linked to speed of absorption and the concentration in arterial blood reaching the brain.

Background: Ethanol is a major drug of abuse and the blood-ethanol concentration (BAC) reached after drinking has important ramifications in clinical and legal medicine. Threshold limits of blood-ethanol concentration exist for driving a motor vehicle, such as 0.05 g/100 mL or 0.08 g/100 mL, which apply in most countries. Accordingly, variability in the rates and extent of absorption, distribution and elimination of ethanol are much discussed and debated during the prosecution of drinking drivers.

Arterial-venous differences: The pharmacokinetics of ethanol were determined in venous (V) and arterial (A) blood in healthy men (n = 9) and women (n = 6). They drank ethanol (0.60 g/kg) on an empty stomach and parameters representing absorption, distribution, and elimination processes were calculated for blood samples obtained through indwelling catheters in radial artery and cubital vein on the same arm. The specimens were taken every 10-15 min for up to 6-7 h post-dosing. Ethanol was determined by headspace gas chromatography. The peak concentration of ethanol in arterial blood was 0.103 g/100 mL (SD 0.022) compared with 0.092 g/100 mL (SD 0.021) in venous blood ($p<0.05$). The mean ascending rate of change in ABAC was 0.171 g/100 mL/h, being faster than 0.138 g/100 mL/h for VBAC ($p<0.05$). The C_{max} for ethanol occurred 5 min earlier in arterial blood than venous blood. The maximum A-V difference in concentration of ethanol occurred 10 min after drinking ended [0.019 g/100 mL (SD 0.0107)], decreasing thereafter as absorption progressed. Between 90-110 min post-dosing, the A-V ethanol difference was zero and at all later times VBAC always exceeded ABAC by 0.0 to 0.01 g/100 mL. The rate of ethanol disappearance from A-blood (k_o) was 0.013 g/100 mL/h (SD 0.0021) compared with 0.012 g/100 mL/h (SD 0.0022) in V- blood ($p<0.05$). The ABAC always reached zero before VBAC (410 min, SD 43) compared with 436 min (SD 47). The distribution of ethanol in the body as reflected in AUC, volume of distribution, and C_o were not significantly different for the A and V sampling sites. Arterial-venous differences in ethanol concentration reflect the uptake of ethanol into tissue water during the loading phase and extraction from tissue water as ethanol is cleared from A-blood during the post-absorptive phase.

Gastric bypass patients: The absorption, distribution, and elimination kinetics of ethanol were studied in 12 healthy women who had undergone gastric bypass surgery for morbid obesity at least three years earlier. As a control group 12 other women closely matched in terms of age and body mass index (BMI) served as controls. All subjects received the same dose of ethanol (0.30 g/kg) mixed with orange juice (20 % v/v). This drink was consumed in 5 min on an empty stomach. Specimens of venous blood were taken from an indwelling catheter before and at 10 min intervals for up to 3½ hours post-dosing. In gastric bypass patients, the peak blood-ethanol concentration was higher (0.074 ± 0.021 g/100 mL vs 0.058 ± 0.011 g/100 mL) with a marked overshoot peak amounting to 62.1% compared with 29.6% in the non-operated control group ($p<0.05$). The peak blood-alcohol concentration was reached 30 min sooner in the gastric bypass women. Beyond 45 minutes post-drinking, blood-alcohol concentration was not significantly different in the two groups ($p>0.05$). The rate of alcohol disappearance from blood was the same in operated and control groups (0.014 g/100 mL/h) although the curves reached zero BAC earlier in bypass patients (211 ± 34 min) compared with controls (227 ± 10 min). The distribution volumes of ethanol were 0.616 ± 0.10 L/kg vs 0.592 ± 0.087 L/kg, in operated and control groups, respectively. The more pronounced feelings of inebriation in gastric bypass patients who consume alcohol can be explained by a transient, but more rapid absorption, of ethanol from the gut.

Short-term fluctuations in blood- and breath-ethanol concentration: Concentration-time profiles of ethanol were determined in venous whole blood and end-expired breath after taking a large number of successive specimens at short intervals. Moderate doses of ethanol (0.3-0.4 g/kg) were administered either orally or intravenously to healthy volunteers. Specimens of venous whole blood were taken from an indwelling catheter at 5-min intervals post-dosing for two hours and then every 10 min for an additional two hours. Ethanol was determined in blood samples by highly reliable method based on headspace gas chromatography having an analytical precision (coefficient of variation, CV) of less than 1%. The concentration of ethanol in breath was determined with the aid of a quantitative infrared analyzer (Datamaster) immediately after blood samples were taken. The analytical precision (CV) of the breath-analyzer was about 2%. When alcohol was taken orally, there was a tendency towards zig-zag BAC profiles during the first one hour after drinking corresponding to the absorption phase. This observation probably reflects intermittent gastric emptying. However, sporadic fluctuations in BAC and BrAC did not exceed more than 0.005 g/100 mL. No evidence was found for major oscillations in BAC or BrAC profiles during the post-peak phase. During this post-absorptive period, the average scatter of data points (residual SD) was ±2% for BAC profiles and ±4% for the corresponding BrAC profiles. The means that the magnitude of differences in replicate measurements with 95% probability (2 x SD) are ± 4% and ± 8% for BAC and BrAC, respectively, and this includes both analytical and biological sources of variation.

Conclusions: The results of these new studies conducted under controlled conditions show the importance of blood sampling site (artery vs. vein) for the underlying pharmacokinetic parameters of ethanol, being especially evident during the absorption phase. In gastric bypass patients the lack of a functioning pyloric sphincter caused more rapid absorption of ethanol into the blood and exaggerated feelings of inebriation, albeit for a short time post-dosing. The existence of marked

short-term oscillations in the time course of blood-and breath-alcohol profiles when successive samples are taken a 5-min interval could not be confirmed.

Alcohol, Pharmacokinetics, Toxicology

K32 Blockade of Physiological and Subjective Effects of Smoked Marijuana by the First CB1-Selective Cannabinoid Receptor Antagonist SR141716

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The objective is to report results of the antagonism of marijuana smoking by the first cannabinoid receptor antagonist, SR141716. New advances in understanding the important role of the endocannabinoid system in cognitive processes, motor control, and appetite will be presented.

Delta-9-tetrahydrocannabinol (THC), the primary psychoactive component of marijuana, produces behavioral, cardiovascular, analgesic, psychomotor, and cognitive effects that are mediated through the cannabinoid CB1 receptor, a G-protein linked receptor, located primarily in the central and peripheral nervous system. A second cannabinoid receptor subtype (CB2) appears to be concentrated in the peripheral immune system. The identification of specific cannabinoid receptors has led to the discovery of endogenous cannabinoid agonists, including the arachidonic acid derivatives anandamide and 2-arachidonoylglycerol. SR141716, a recently developed CB1-cannabinoid receptor antagonist, blocks acute effects of THC and other CB1 cannabinoid agonists in vitro and in animals and precipitates withdrawal signs in animals treated chronically with THC. These findings suggest that CB1 receptors mediate many of the effects of marijuana, but this has not been evaluated in humans.

Sixty-three healthy males (mean [SD] age 27.7 [5.4] years, 70% African-American, 10.3 [5.9] years of lifetime marijuana use, 15.3 [10.2] days of marijuana use in the prior month) participated in the research study. Their health status was evaluated by medical history, physical examination, blood chemistries, complete blood count, urinalysis, viral antibody screening (hepatitis B, hepatitis C, HIV), TB skin testing (with chest X-ray as clinically indicated), electrocardiogram, EEG, pulmonary function tests, clinical psychiatric interview, and standard psychological tests. To avoid the possibility of precipitating marijuana withdrawal, SR141716 was not administered until urine cannabinoid concentration was below 20 ng/mL (EMIT II Cannabinoid 20 ng Assay, Behring Diagnostics, Inc., Cupertino, CA). The study was approved by the NIDA Institutional Review Board. All subjects gave written informed consent and were paid for their participation. Subjects were randomly assigned to receive a single oral dose of SR141716 or placebo double blind in an escalating dose (1, 3, 10, 30, 90 mg) design. An active (2.64 % THC) or placebo marijuana cigarette was smoked two hours after oral SR141716. Subjects' subjective response to marijuana was assessed before and after SR141716 and marijuana administration using 100-mm visual analog scales (VAS) and the marijuana subscale of the Addiction Research Center Inventory (M-scale). Heart rate and blood pressure were measured prior to, during, and after SR141716 and marijuana administration.

Marijuana smoking produced the expected cardiovascular effect of tachycardia. There was a significant orthogonal polynomial linear trend for SR141716 dose-dependent blockade of marijuana-induced tachycardia. The 90-mg SR141716 dose reduced tachycardia by 59% and reduced symptomatic episodes of hypotension to 0% of marijuana-smoking sessions (vs. 20% incidence on placebo SR141716). Marijuana produced expected psychological responses reflecting intoxication. The 90 mg dose of SR141716 produced significant (38 to 43% reductions) dose-dependent blockade of marijuana effects (as compared to placebo SR141716) on composite VAS (t=2.23, df=47, P=0.03) and individual VAS (t=2.49, df=47, P=0.02; t=1.97, df=47, P=0.05; and t=2.37, df=47, P=0.02 for "How high do you feel now?," "How stoned on marijuana are you now?," and "How strong is the drug effect you feel now?," respectively) and produced a trend (a 75% reduction) toward reduced M scale peak effects (t=1.97, df=47, P=0.06). Orthogonal polynomial trend tests indicated a significant linear trend for SR141716 dose on composite VAS ($F_{1,47}$=5.59, P=.02) and M scale ($F_{1,47}$=6.22, P=.02). SR141716 alone produced no significant effect on heart rate or blood pressure or psychological effects and did not affect peak THC plasma concentration or area under the time X concentration curve. SR141716 was well tolerated by all subjects. No subject left the study because of adverse events related to SR141716. There was no difference in frequency of occurrence of adverse events between the 43 subjects receiving active SR141716 and the 20 subjects receiving placebo SR141716. These findings show a direct pharmacodynamic blockade of smoked marijuana effects by SR141716, with no evidence of pharmacokinetic interaction. They confirm for the first time the essential role of CB1 receptors in mediating the cardiovascular and subjective effects of smoked marijuana in humans. SR141716 was well tolerated, suggesting it may be a useful tool for studying the function of the endogenous cannabinoid system in humans.

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Marijuana, Cannabinoid Receptor, Antagonist, SR 141716

K33 Evaluation of a Solid Phase Extraction Method for BZE Urinalysis in a High Throughput Forensic Drug Testing Laboratory

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Attending this presentation, the participant will learn technical details of a novel urinalysis procedure for benzoylecgonine. The results of a validation and advantages of the method will be discussed.

Although cocaine use has been steady or declining in recent years, the international trafficking network remains intact and has thwarted numerous attempts to limit importation (1). Adding to the threat, cocaine is sold on the street at lower cost and higher purity despite the coordinated efforts of law enforcement. Consistent with these trends, cocaine use has been a constant concern for the United States Navy. In 1980, the Department of Defense (DoD) established a zero-tolerance drug policy to deter the use of illicit drugs. The program has been very successful, and currently, the U.S. Navy tests approximately 1.6 million urine samples per year. To meet the high sample volume, a novel extraction and derivitization procedure for a major cocaine metabolite, benzoylecgonine (BZE), was developed and evaluated for use in a high-volume forensic urinalysis laboratory.

Urine specimens were spiked with the internal standard ($^{2}H_{8}$ Benzoylecgonine) and extracted utilizing a Speedisk 48 positive pressure extraction manifold and polymer based cation exchange extraction column. Samples were derivitized by the addition of pentafluropropionic anhydride (PFPA) and pentafluoropropanol (PFPOH) (2). All confirmation testing was performed by Gas Chromatography/Mass Spectrometry (GC/MS) in the single-ion monitoring mode (SIM); ions included m1z 421, 300, 272, 429, 303 with m/z 421/429 used for quantitation.

The validation protocol included the determination of sensitivity and linearity, an interference study, and statistical analysis. Sensitivity and linear range were established utilizing twenty samples spiked at the cutoff concentration of 100 ng/ml and thirteen concentration levels ranging from 12.5 to 20,000 ng/ml. The limit of detection was determined to be 12.5 ng/ml; the limit of quantitation was at 12.5 ng/ml, and the assay was linear from 12.5 to 20,000 ng/ml with an r^2 of 0.99932 (F=38319, df=27). Excellent correlation between measured and expected results was observed over a wide range of concentrations. The interference study consisting of a series of 100 ng/mL control samples spiked with 74 common medications demonstrated no significant chemical interferences.

The method was further validated by comparison to the analytical procedure currently employed by the laboratory for the reporting of positive cocaine urinalysis results. Specifically, a set of 79 archived samples, previously reported positive for BZE, were extracted, analyzed, and compared to the previously reported quatitations. In addition, aliquots of seventeen of the 79 samples were assayed independently at the Navy Drug Testing Laboratories in San Diego CA, and Great Lakes IL. Results between the three laboratories and prior quantitations were analyzed by one-way ANOVA. The BZE results were not significantly different between laboratories nor were they significantly different from the prior quantitations (F=0.05309, P=0.99463).

The new assay is reproducible in comparison to previous methods used in our laboratory. Since implementation, the new procedure has been pivotal in improving laboratory efficiency, and a significant improvement in turn-around-time has been observed. The procedure will provide a reliable and efficient procedure for BZE urinalysis.

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Urinalysis, Solid Phase Extraction, Cocaine

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