Author Index

Bolded type denotes presenting author.

Bolded type denotes presenting author.

Bolded type denotes presenting author.

Bolded type denotes presenting author.

Bolded type denotes presenting author.

Bolded type denotes presenting author.

Keyword Index

Abstracts Of Platform Presentations

S01

Comparison of Pentobarbital Recovery in Decomposed Bone Following Ultrasonic-Solvent Extraction and Standard Passive Extraction and Gas Chromatography-Mass Spectrometry

Caroline C. Betit*[1], James H. Watterson[1], Dorothy E. Dean[2], John F. Wyman[3]; [1]Departments of Forensic Science, Chemistry & Biochemistry, Laurentian University, Sudbury, Ontario, Canada, [2]Summit County Medical Examiner's Office, Akron, OH, USA, [3]Cuyahoga County Regional Forensic Science Laboratory, Cleveland, OH, USA

Introduction: Although a novel technique, toxicological analysis of skeletal tissue has gained increasing attention in recent years. Unfortunately, standard passive solvent extraction requires extensive incubations, thus limiting analytical throughput. Accordingly, a methodology for rapid ultrasonic solvent extraction of pentobarbital from decomposed bone tissue was developed and compared to a standard passive extraction approach.

Method: Bone was derived from remains of a domestic pig exposed to diazepam (7.5 mg/kg), citalopram (7 mg/kg), amitriptyline (75 mg/kg), morphine (0.8 mg/kg) and euthanized with an injection of PB (30 mg, IP) followed by a lethal intracardiac injection of PB (390 mg). After euthanasia remains decompose to skeleton in a secure outdoor environment (rural Ohio) for over 2 years. Bones were recovered and separated according to their anatomical location, washed with phosphate buffer (PBS, 0.1 M, pH 6), methanol and acetone and dried under ambient conditions and pulverized with a domestic grinder. Drug-free pig bones were used as negative control. Ground vertebrae were sampled in triplicates where different variables, such as sample mass (1-2 g), solvent polarity (methanol vs. ethyl acetate), solvent volume (5, 10 and 15 mL) and extraction time were investigated. The extraction solvent was replaced with fresh solvent after 24, 48, 72, 96 h for passive extraction (50 °C) and 10, 30, 45, 60, 90 min for ultrasonication. Samples were evaporated to 1 mL and the volume was then adjusted to 4 mL with PBS. Secobarbital (SB, 50 ng) was added as an internal standard. A lipid/protein precipitation step with 1:1 acetonitrile: methanol (3 mL) preceded mixed-mode solid phase extraction (SPE). Columns were conditioned with methanol, water and PBS and samples were loaded by gravity. Columns were washed sequentially with PBS and 0.1 M acetic acid before PB was eluted with 3 mL of methanol. Extracts were evaporated to dryness and reconstituted in ethyl acetate (EA) and derivatizing agent (TMPAH) was added before analysis. GC/MS analysis was done in the SIM mode, where ions monitored were m/z 112, 169 and 184 (PB) and 181,195 and 196 (SB). This assay has a detection limit of approximately 5 ng/mL PB (based on 1 mL initial solution). Mass-normalized response ratio (RR/m) was used for all statistical analysis (ANOVA one-way with post-hoc testing).

Results: Results indicate a significant difference between the two solvents for both passive and ultrasonic extraction, where a higher yield was observed with methanol. A significantly higher recovery was also observed with 2 g samples for both extraction methods. Solvent volume did not significantly influence analyte yield via ultrasonication, but increasing the extraction solvent volume to 15 mL proved to increase the yield significantly for passive extraction. Ultrasonication resulted in significantly faster extractions, with maximum yields obtained roughly 60-fold faster than that was observed using using passive solvent extraction.

Conclusion: Finally, the comparison between the two extraction methods resulted in a significant difference in yield for both 1 g and 2 g when using samples extracted in methanol and 2 g samples when samples were extracted in EA. In all three cases passive extraction produced higher yields. For qualitative and semi-quantitative work, ultrasonic solvent extraction is proven to be more cost and time efficient.

Keywords: Forensic Toxicology, Bone, Ultrasonication, Drug, Pentobarbital, GC-MS

S02

Updating Methods: How to Efficiently Streamline Four Opiate Methods into One

Dani C. Mata*, Bernard Huettl; OC Crime Lab, Santa Ana, CA, USA

Introduction/Objective: Opiates are one of the most commonly abused drugs in the nation when it comes to driving under the influence of drugs and overdoses. Previously at the Orange County Crime Lab, four different methods were used to determine the presence and concentration for six free and total opiates; morphine, codeine, hydrocodone, hydromorphone, oxycodone, and oxymorphone. These methods were all liquid-liquid extraction and quite time intensive. In order to determine the opiate concentration, multiple methods would have to be used, using much of the limited supply of blood.

Method: A solid phase extraction (SPE) method was developed to analyze for the six previously mentioned opiates and 6-monoacetylmorphine (6-MAM) in both free and total concentrations by GC/MS in one extraction. In this method, six standards are extracted with the samples, all which include an internal standard of the D3-opiate for each of the 7 opiates for quantitation. The oxime derivative of the keto-opiates (hydrocodone, hydromorphone, oxycodone, and oxymorphone) is formed by adding 5% methoxylamine. This is done to prevent derivatization of the enol isomer with the propionic anhydride, which would produce two products. Once the oxime has formed, an automated SPE is performed and then the opiates are derivatized for 30 minutes at 100^{o}C with pyridine and propionic anhydride to convert the hydroxyl groups on the 3-carbon to the propionyl ester. Two ion ratios for each opiate are used for identification by GC/MS. Should a sample need a total opiate extraction, a hydrolysis standard is included and all samples requiring total analysis are hydrolyzed using a *β-Glucuronidase Type HP-2 from Helix pomatia* enzyme for three hours at 40-50^{o}C.

Results: Possible carry-over issues on the SPE instrument and GC/MS were addressed during development, along with matrix effects, possible interferences, and stability for all seven opiates. The matrix effects were evaluated by extracting spiked liver, stomach contents, urine, blood, and brain samples. Once the comprehensive method was developed, the limit of quantitation (LOQ), limit of detection (LOD), and limit of linearity (LOL) were determined from running standards of each drug at various concentrations at least ten times. Table 1 has these values along with the coefficient of variation (CV), which was determined by tracking the quality control concentration for 90 runs.

Table 1: LOQ, LOD, LOL, and CV for each opiate in all matrices

	LOQ (µg/mL)	**LOD (µg/mL)**	**LOL (µg/mL)**	**CV (%)**
Morphine	0.02	0.01	0.02 - 5	9.3
Codeine	0.02	0.005	0.02 – 5	8.4
6-MAM	0.01	0.01	0.01 - 5	8.6
Hydrocodone	0.01	0.01	0.01 - 5	8.6
Hydromorphone	0.01	0.005	0.01 – 5	10.4
Oxycodone	0.02	0.01	0.02 – 5	11.7
Oxymorphone	0.005	0.0025	0.005 – 2.5	8.3

Conclusion: Once developed, the method was validated in blood, urine, brain, liver, and stomach contents before being put into use at the laboratory. For the free opiate validation 368 samples were examined with only 9 samples deviating more than 20% from the originals. It was determined that these nine samples had lost the opiate of interest due to adherence to glass since the first analysis occurred many months before the validation analysis. For the total validation 153 samples were examined and none were outside of 20% of the original concentration. A cost comparison of the old and new method will also be examined in this presentation.

Keywords: Opiates, Method Development, Solid Phase Extraction

S03
The Effect of Burn Injury on Blood Ethanol and Ethyl Glucuronide Concentrations

Trista H. Wright*, Kenneth E. Ferslew; Section of Toxicology, East Tennessee State University, Johnson City, TN, USA

Introduction: Alcohol is the most abused drug in the United States and is present in biological specimens from fatal residential house fire victims in 40% of all fires. There is no known data available to validate blood alcohol concentrations and their interpretations in fire-related death victims.

Objective: Determine if exposure to a house fire causes changes in post-incineration blood ethanol and ethyl glucuronide (EtG) concentrations from pre-incineration concentrations.

Methods: A Sprague Dawley rat model was used to determine the effect of burn injuries, using two fire-related models, on blood ethanol and ethyl glucuronide (EtG) concentrations. One hundred twenty six male rats were gavaged orally with 4 g/kg of ethanol then placed in metabolic cages for three hours until euthanization. Burn injuries from fire deaths were mimicked using the reported average response time by local fire departments and two types of incineration, using a flame and thermal incineration, with these conditions:

Flame Incineration (Fire Pit) (n=9 per group, 3 groups) Temperature: > 1000°F	Duration (minutes): 2, 5, and 8
Thermal Incineration (Gas Grill) (n=9 per group, 9 groups) Temperature: 200, 400, or 600°F	Duration (minutes): 2, 5, or 8

Pre-incineration and post-incineration heart bloods were analyzed for ethanol by gas chromatography and EtG by enzyme immunoassay to determine if any differences occurred in analyte concentrations due to flame and/or thermal incineration. Additional chest blood specimens were collected when available and analyzed for ethanol and EtG. Core body temperatures were monitored using a rectal probe to determine if a correlation existed between changes in analyte concentrations and maximum core body temperature.

Results: Results show that blood ethanol concentrations were not effected but EtG concentrations were elevated in groups from both models ($\rho < 0.05$). When extra available chest blood was substituted for heart blood the ethanol concentrations significantly increased in thermal incineration groups with higher time/temperature exposures and remained unchanged in other flame and thermal incineration groups with additional available chest blood. Maximum core body temperatures ranged from 96-151°F for incineration groups.

Conclusion/Discussion: An elevated post-incineration EtG concentration may cause misinterpretation when ethanol to EtG ratio is used to predict prior ethanol consumption behavior. Neither burn injury model produced statistical significant increases or decreases in post-incineration blood ethanol concentrations compared to their pre-incineration levels. Our research suggests caution should be used when interpreting ethanol impairment using specimens of either unknown origin or composition, or which have been collected when there is loss of integrity to the vascular compartment from burn injury.

Keywords: Forensic Science, Ethanol, Ethyl Glucuronide, Burn Injury, Postmortem, Blood

S04

What is the Best Biological Matrix for Identifying Methamphetamine Environmental Exposure in Endangered Children?

Marisol S. Castaneto*[1], Allan J. Barnes[1], Karl B. Scheidweiler[1], Michael Schaeffer[2], Kristen K. Rogers[3], Deborah Stewart[3], Marilyn A. Huestis[1]; [1]Chemistry and Drug Metabolism, NIDA-IRP, NIH, Baltimore, MD, USA, [2]Psychemedics, Corporation, Culver City, CA, USA, [3]California Department of Public Health, Sacramento, CA, USA

Background: The Child and Adolescent Abuse, Resource, Evaluation (CAARE) Diagnostic and Treatment Center at UC Davis, performed medical evaluations on approximately 150 children per month to determine the need for protective custody; about 70% involved exposure to illicit drugs in homes with the majority being methamphetamine - whether manufactured, used and/or sold. However, unless the child's urine is positive for a drug, substantiating exposure, the child is frequently returned to their previous drug-exposed environment.

Objectives: To determine the most effective matrix for identifying children exposed to methamphetamine (MAMP), amphetamine (AMP), MDMA, MDA, and/or MDEA by comparing detection rates in concurrently collected urine, hair and oral fluid (OF) specimens.

Methods: An IRB-approved study was conducted on 248 de-identified specimens collected from 91 children examined at CAARE. Urine (86) and OF (72) collected with the Orasure device were analyzed by GCMS for MAMP, AMP, MDMA, MDA and MDEA at the National Institute on Drug Abuse (NIDA). Linear ranges were 25-5000 ng/mL and 15-1500 ng/mL for all analytes in urine and OF, respectively. Proximal segments (10mg) from 90 hair specimens were evaluated by Psychemedics, Corp. for MAMP; MDMA and MDEA (1ng/10mg), AMP (0.25ng/10mg), MDA (0.2ng/10mg) by tandem LCMSMS.

Results: MAMP (1-168ng/10mg), AMP (0.25-11.5ng/10mg), and MDMA (1-22.8ng/10mg) were detected in 67, 50 and 11 hair specimens, respectively. AMP was present with concurrent MAMP and at lower concentrations in all but one specimen, while MDMA was detected in the presence of MAMP in 9 of 11 hair specimens. Considering a hair specimen positive if MAMP, AMP or MDMA concentrations were >LOQ produced an overall detection rate of 78.8%. MAMP was the primary analyte in 18 urine specimens at concentrations <108ng/mL, although AMP was detected in fewer specimens (3) concentrations ranged from 25.2-3581ng/mL. Urinary AMP was detected without concurrent MAMP in one specimen, increasing the overall positive detection rate to 22.1%. Only 5 of 72 OF specimens tested positive for MAMP (17.9-133.6ng/mL). AMP was present (21.4ng/mL) only in the specimen with the highest MAMP concentration. Overall amphetamines detection rate for Orasure OF was 6.9%. All positive OF (n=5) and 18 of 19 positive urine specimens also had a positive hair test. All matrices were available for 67 children, 12 were negative and 2 were positive in hair, OF and urine, and 53 were positive in one or more matrices. MDA and MDEA were <LOQ in all matrices.

Conclusion: Urine and OF testing can detect recent MAMP, AMP and MDMA exposure. However, hair testing analysis was most suitable for detecting drug exposure over a larger timeframe. A negative urine, OF or hair test cannot ensure the absence of drug exposure, as minimal limits of detection, loss of drug from hair over time and many other factors are not established. Hair analysis offers a more sensitive tool for detecting MAMP, AMP and MDMA exposure in endangered children than urine or OF testing.

Funded by the Intramural Research Programs, National Institute on Drug Abuse and Mental Health, National Institutes of Health.

Keywords: Hair, Urine, OF, Drug-Exposed Children, Methamphetamine, Amphetamine, MDMA

S05

The Trouble with Tramadol: Can Accurate Mass Alone Provide an Identification?

Roman P. Karas*, Louis J. Reda, Rena L. Hammer; FBI Laboratory, Chemistry Unit, Quantico, VA, USA

Introduction: In recent years, the use of advanced liquid chromatography mass spectrometry (LC/MS) has become more commonplace in routine forensic toxicological examinations. Tandem liquid chromatography mass spectrometry (LC/MS/MS) provides additional specificity through the fragmentation of the pseudo-molecular ion. Accurate mass techniques, such as Fourier-transform mass spectrometry (FTMS) and time of flight mass spectrometry (TOF-MS) are also gaining more acceptance in the community. Today's technology does provide the practicing toxicologist a wealth of information that was unavailable even a decade ago. However, it is still prudent to exercise caution when dealing with unusual situations or unfamiliar drugs and metabolites; as misidentifications can still occur.

Objective: The presentation will describe the analysis of a routine post-mortem case which presented an initially conflicting set of analytical results. The analytical scheme used by our laboratory will be described, highlighting the strengths and weaknesses of the techniques employed. The resolution of the apparent discrepancy will be described.

Methods: The case was analyzed by a ELISA, gas-chromatography mass spectrometry, LC/MS/MS, and LC/FTMS.

Results: The initial immunoassay indicated a presumptive high presence of phencyclidine (PCP) as well as benzodiazepines and opiates. Other drugs initially identified through GC/MS and LC/MS screens included venlafaxine; norvenlafaxine; atropine; diazepam; nordiazepam; oxycodone; and tramadol. Quantitation was successful for the relevant drugs; with the exception of PCP and tramadol. The lack of PCP confirmation could be traced to cross reactivity with venlafaxine on the immunoassay. Literature references provided some explanation for this result. The lack of tramadol confirmation proved to be more complex. Through a combination of limited reanalysis and research, it was determined that tramadol shares the same empirical formula with the primary metabolite of venlafaxine, o-desmethylvenlafaxine. As such, the compounds were not resolvable by accurate mass alone. Additionally, tramadol and o-desmethylvenlafaxine share similar LC retention times and fragment identically under the analysis conditions used. However, by using a different chromatography system, the retention times for the two analytes were rendered distinguishable. Therefore, through consideration of the total analytical picture, correct identification of the compounds was readily achieved

Discussion: Even with the array of advanced instruments available to today's toxicologist, a working knowledge of available drugs, drug metabolism, and the limitations of the instruments employed is critical to preserving analytical integrity. Although tandem mass spectrometry and accurate mass spectrometry do offer high specificity, use of these techniques alone does not guarantee correct identification.

Keywords: Accurate Mass, Tandem Mass Spectrometry, Specificity, Tramadol

S06
A Drug Fatality Involving Kratom: A Case Report

Randall E. Frost, Janine Deking, **Michael F. Neerman***, Bexar County Medical Examiner's Office, San Antonio, TX, USA

Background: A 17-year-old white male who showed no signs of obvious trauma was found unresponsive in bed and was pronounced dead at the scene. The decedent had a documented history of heroin abuse and chronic back pain and reportedly self-medicated with Kratom (mitragynine) that he had acquired online.

Objective: The objective of this case report was to determine whether Kratom (mitragynine) could have played a role in the cause of death.

Methods: A full autopsy was performed by the medical examiner. Femoral blood was collected and subjected to a full toxicology work up. Given the circumstances surrounding the case, mitragynine analysis was performed utilizing tandem liquid chromatography MS/MS. Briefly, the system used was an Agilent 1100 Series Liquid Chromatography coupled to an Applied Biosystems/MDS Sciex 3200 QTRAP MS/MS utilizing a C18 analytical column. The analysis was done in MRM (multiple reaction monitoring) mode monitoring transitions 399.2/174.2 and 399.2/151.9.

Results: The autopsy was remarkable only for pulmonary congestion and edema and a distended bladder, both of which are consistent with, though not diagnostic of, opiate use. A laboratory work-up revealed therapeutic levels of over-the-counter cold medications such as dextromethorphan and diphenhydramine. Temazepam and 7-aminoclonazepam were also detected. However, of interest, was the mitragynine blood concentration at 0.6 mg/L.

Conclusion: The case we are presenting involves a possible death resulting from Kratom use. Kratom (Mitragyna speciosa;) is a leafy tree indigenous to southeast Asia and has been traditionally used for its purported medicinal properties. Kratom can be obtained through online sources and “head shops” and is used as a remedy for opioid withdrawal as well as recreational use. The effects of Kratom are attained by the predominant psychoactive substances mitragynine and 7-hydroxymitragynine. Unfortunately there is a paucity of case reports publishing blood drug concentrations in Kratom/mitragynine fatalities. In addition, lethal blood drug concentrations have not been established. Given the facts of the case, the Medical Examiner certified the cause of death as “possible Kratom toxicity" and the manner of death was classified as “accident."

Keywords: Kratom, Mitragynine, Overdose, Forensic Toxicology

S07
Opiate Chemistry and the Toxicologist - A Global Overview

Minh T. Dang*; United States Drug Enforcement Administration, Washington, DC, USA

Introduction: According to the 2011 White House Report entitled, "Epidemic: Responding to America's Prescription Drug Abuse Crisis", prescription drug abuse is the Nations's fastest-growing drug problem. According to this report, from 1997 to 2007 the milligram per person use of prescription opioid use in the U.S. has increased from 74 mg to 369 mg, an increase of 402 percent. In addition, in 2000, retail pharmacies dispensed 174 million prescriptions for opioids. By 2009, 257 million prescriptions were dispensed, an increase of 48 percent. Opiate overdoses, once almost always due to heroin use, are now increasingly due to abuse of prescription opiates.

Objective: This presentation will provide the toxicologist a global overview of the science, chemistry, synthetic routes and regulatory controls for opium based products.

Methods: In addition to discussing the illicit clandestine manufacture of heroin, this presentation will discuss the many drug products derived from the opium poppy alkaloids oripavine, thebaine, codeine and morphine. This presentation will discuss structural similarities of the opiates and will review and trace the various chemical transformations of opium from its chemical precursors; to its various chemical intermediary's; to its final products as bulk active pharmaceutical ingredient (API).

Results: Upon understanding the chemical transformations of opium to bulk active pharmaceutical ingredient (API), we will discuss the end products as finished dosage units such as the prescription drugs oxycodone, hydrocodone, hydromorphone, oxymorphone, buprenorphine, morphine, diprenorphine, ethylmorphine, noroxymorphone, naloxone, naltrexone, nalbuphine and as chemical preparations used by the toxicologist such as immunoassay kits; drug standards; and as DEA exempt chemical preparations.

Conclusion: Knowledge of the manufacturing process, chemical transformations, pharmaceutical products and toxicological products derived from opium will provide the toxicologist a global overview and greater understanding of the movement and transformation of opiates from poppy to pipet.

Keywords: Opiates, Heroin, Prescription Drugs, Opium

S08

Post-Mortem Redistribution of Drugs: A Literature Review and Statistical Study of Data for Venlafaxine, Citalopram, Trazodone and Cocaine

Brigitte Desharnais*[1], Félix Camirand-Lemyre[2], Pascal Mireault[3]; [1]Department of Toxicology, Laboratoire de Sciences Judiciaires et de Médecine Légale, Montréal, (Québec) Canada, [2]Department of Mathematics, Université de Sherbrooke, Sherbrooke, (Québec) Canada, [3]Department of Toxicology, Laboratoire de Sciences Judiciaires et de Médecine Légale, Montréal, (Québec) Canada

Introduction: Post-mortem drug redistribution (PMR) is commonly defined as the site and time dependent change in drug concentration after death. Knowledge of the extent of PMR is important to forensic toxicologists in order to correctly interpret post-mortem drug concentrations. The extent of PMR is typically evaluated by the ratio of the drug concentration in central blood to the concentration in peripheral blood; which is considered to represent more accurately the ante-mortem concentration.

Objectives: Studies on PMR published in the past have been indicative but suffer from small sample size and/or flaws in the statistical analysis process. This work aimed to produce a larger-scale study of PMR of venlafaxine, citalopram, trazodone and cocaine using more appropriate statistical methodologies. The specific points of inquiry were the existence of a PMR phenomenon, the importance of diffusion from gastric content as a PMR mechanism, the influence of cause of death, putrefaction and post-mortem interval, coherence of results with pharmacodynamic properties and the potential of PMR to differentiate drug induced death and other causes of death.

Methods: All cases reported in the Montréal's Forensic Laboratory files in which venlafaxine, citalopram, trazodone or cocaine was found since 1995 were compiled. Statistical methods appropriate to study the extent of PMR and its correlation with the different factors were defined and applied to the data set of cases (62 to 214) for the four drugs. Briefly, Student's T-tests and ANOVA were used depending on the questions with an $\alpha=0.05$ (95% confidence) as the threshold for significance.

Results: A significant difference between the concentrations found in the cardiac blood and in the femoral found existed for venlafaxine, citalopram and cocaine. This difference was not affected by gender or age. A correlation between the cardiac to femoral concentration ratio and the quantity of drugs present in the gastric content was found to exist for venlafaxine and citalopram. For all drugs studied, the cardiac to femoral concentration ratio was not found to be significantly different for different causes of death (gunshot, multiple trauma, intoxication, drowning, aphyxia, cardiac illness). The cardiac to femoral concentration ratio was found to be different depending on the degree of putrefaction of the body for cocaine, but not for venlafaxine and citalopram. On the other hand, the cardiac to femoral concentration ratio was not found to differ according to the post-mortem interval for venlafaxine, trazodone and cocaine, but was found to differ for citalopram. Finally, the ratio was not found to be significantly different for intoxication and other causes of death for citalopram and cocaine.

Conclusions: These results indicate the existence of post-mortem redistribution for venlafaxine, citalopram and cocaine. Diffusion from the gastric content into the blood seems to be an important mechanism for venlafaxine and citalopram. Processes specific to cause of death; such as haemorrhages do not seem to have an impact on the extent of PMR. There does not seem to exist a potential to distinguish between drug induced death and other cause of death using the cardiac to femoral concentration ratio.

Keywords: Post-Mortem Redistribution, Statistical Analysis, Gastric Content, Central to Peripheral Blood Concentration Ratio, Venlafaxine, Citalopram, Trazodone, Cocaine

S09
A Wide Range of Circumstances Observed in MDPV-Related Deaths

Lauren L. Richards-Waugh*, Christina L. Newsome-Sparks, Kristen M. Bailey, Myron A. Gebhardt, David J. Clay, James C. Kraner; Office of the Chief Medical Examiner, Charleston, WV, USA

Introduction: In 2011, the news media throughout the United States was replete with stories about bizarre and often violent behavior attributed to the recreational use of a group of compounds that had acquired the name “bath salts". Users purchased these products through numerous online websites, gas stations, and convenience stores. Many contained the synthetic cathinone derivative 3,4-methylenedioxypyrovalerone (MDPV). With structural similarity to methcathinone, methamphetamine, and MDMA, MDPV users typically experience similar, significant behavioral effects such as hallucinations coupled with central nervous system stimulation. Beginning in March of 2011, the West Virginia Office of the Chief Medical Examiner (WV OCME) began receiving deaths involving the use of MDPV, which has been detected in 11 deaths investigated in our state.

Objective: The objective of this presentation is to discuss the various circumstances of death observed in MDPV-related cases investigated by the WV OCME.

Methods: Routine toxicological analysis included a standard battery of tests- blood alcohol by GC-FID, drugs of abuse enzyme immunoassay, and GC/MS screening of alkaline and acidic/neutral blood extracts. GC/MS analysis of urine extracted using Toxi-Lab A tubes was also beneficial in identifying the presence of MDPV. MDPV was confirmed and quantitated by GC/MS analysis of an alkaline liquid-liquid extract.

Results: Of the eleven cases, eight were male and three were female. Decedents ranged in age from 22 to 55. The average blood and urine MDPV concentrations were 0.47 mg/L (0.01-1.09 mg/L) and 13.4 mg/L (0.08 to 60.3 mg/L), respectively. Only one of the 11cases was an isolated MDPV fatality. In several deaths, the decedent was documented to exhibit bizarre behavior such as running away from imagined threats, self-mutilation, and destroying walls and furniture at their place of residence.

Discussion: Similar to a report by Marinetti et al. (1), the circumstances of the MDPV-related deaths investigated by our office are highly variable. Causes of death included overdose, suicide, drowning, motor vehicle accident, and shooting by law enforcement. No MDPV-positive cases have been identified by our office since October 21, 2011 when three synthetic cathinone compounds, including MDPV, were temporarily placed into Schedule I of the Controlled Substances Act (CSA).

References: L. Marinetti; H. Antonides; and J. Watson. More on Bath Salts. ToxTalk 35(3): 11-13 (2011).

Keywords: MDPV, Bath Salts, Synthetic Cathinones

S10

Oral Fluid as an Alternative Matrix to Monitor Buprenorphine Compliance, and Opioids, Cocaine and Tobacco Use in Opioid-Dependent Pregnant Women

Marta Concheiro*[1], Hendree E. Jones[2], Rolley E. Johnson[2,3], Robin Choo[4], Marilyn A Huestis[1]; [1]Chemistry & Drug Metabolism, IRP, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD, USA, [2]Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD, USA, [3]Reckitt Benckiser Pharmaceuticals, Inc., Richmond, VA, USA, [4]Department of Biology, University of Pittsburgh, Titusville, PA, USA

Introduction: Oral fluid (OF) is a promising alternative matrix for detecting recent drug consumption. Compared to urine, OF sample collection is easier, gender-neutral, less invasive and fully observed. However, limited OF drug disposition data are available for opioid-dependent pregnant women.

Objective: To describe buprenorphine (BUP), opioids, cocaine and tobacco prevalence and concentrations in OF from opioid-dependent pregnant women, and to compare detection of opioid and cocaine use in OF and urine.

Methods: Thrice weekly OF and urine specimens (n=446) were collected from 9 opioid-dependent women during the 2nd and primarily the 3rd trimester, and up to 2.5 months postpartum. OF specimens were obtained with the Salivette® collection device, and analyzed by liquid chromatography-tandem mass spectrometry for BUP, norbuprenorphine (NBUP), methadone, 2-ethylidene-1,5-dimethyl-3-diphenylpyrrolidine (EDDP), cocaine, benzoylecgonine (BE), ecgonine methyl ester (EME), anhydroecgonine methyl ester (AEME), morphine, codeine, 6-acetylmorphine (6AM), heroin, 6-acetylcodeine (6AC), nicotine, cotinine and trans-3'-hydroxycotinine (OH-cotinine) (LOQ 0.5-1ng/mL). Urine specimens were assayed for cocaine and opiates by immunoassay (cutoff 300ng/mL).

Results: Women received 8-24mg BUP daily. BUP was detected in 87% of OF specimens (median 440ng/mL; range 0.5-15,700) and NBUP in 66% (6ng/mL; 1-1,800). BUP and NBUP were detected in 64%, but BUP alone in 23%. Weak but statistically significant correlations were found between BUP and OF pH (r=-0.275; P=0.000; n=441); and between NBUP and BUP dose (r=0.132; P=0.006; n=435). Nicotine was detected in 87% of OF specimens (median 216ng/mL; range 1-15,400); cotinine in 86% (108ng/mL; 0.5-733) and OH-cotinine in 71.5% (44ng/mL; 0.6-485). Most OF specimens contained all 3 analytes (69%). Methadone from non-prescribed sources was detected in 13.5% specimens (range 1-1,320ng/mL); and EDDP in only 3 cases (1, 11 and 418ng/mL). Morphine was identified in 16% of specimens (range 0.8-1,070ng/mL), generally alone (9% cases), or in combination with codeine (2.5%) or 6AM (2%). 6AM was present in 7% (0.7-2,580ng/mL), codeine in 4.5% (0.5-37ng/mL), heroin in 2% (1-2,900ng/mL) and 6AC in just 4 cases (0.5-115ng/mL). For identifying illicit cocaine exposure, BE was identified in 44% of specimens (range 0.5-384ng/mL), cocaine in 25% (0.5-419ng/mL), EME in 5% (2-286ng/mL) and AEME in 4% (1-7ng/mL). BE was detected alone in 24% cases, cocaine and BE in 14%, and cocaine only in 5%. For all analytes, drug/metabolite patterns and prevalence encountered in OF were similar during pregnancy and postpartum. There was an 88.5% concordance for any opioid analyte in OF and urine, while for cocaine, only 66% agreement was achieved, with 33.5% positive for any cocaine analyte in OF but negative in urine.

Conclusion: These results offer new information about drug and metabolite concentrations and prevalence in OF from opioid-dependent pregnant women. OF is a good alternative matrix to urine for monitoring drug use also in clinical settings.

Supported by the National Institutes of Health, Intramural Research Program, NIDA.

Keywords: Oral Fluid, Pregnant Women, Buprenorphine, Drug Testing, Saliva

S11
The Effects of Cannabis on Driving Skills

Rebecca L. Hartman*, Marilyn A. Huestis; Chemistry and Drug Metabolism, Intramural Research Program, National Institute on Drug Abuse, NIH, Baltimore, MD, USA

Background: Cannabis, the most prevalent illicit drug identified in impaired drivers, is frequently consumed with alcohol. Despite decades of research, cannabis effects on driving performance continue to be debated, making prosecution and legislation difficult, particularly in states with medical marijuana. Delays in specimen collection, evaluating inactive Δ9-tetrahydrocannabinol (THC) metabolite 11-nor-9-carboxy-THC (THCCOOH), and polydrug use historically complicated epidemiological evaluation of driver impairment.

Objective: To review relevant published data and evaluate current knowledge of cannabis effects on driving.

Methods: PubMed was initially searched with no time restriction for keywords 'cannabis', 'marijuana', 'automobile driving', 'accidents, traffic', and 'motor vehicles'. Subsequent searches included PubMed, Scopus, EndNote, Web of Science, and Embase databases, and additional articles selected from references in previously identified sources.

Results: Epidemiology: Risk of involvement in a motor vehicle accident (MVA) significantly increased after cannabis consumption (point estimate odds ratio [OR], 2.66 [Li, EpiRev 2012]). Adjusting for confounders such as demographics, other drug use and risk-taking behavior attenuated this effect (adjusted point estimate OR, range: 0.8 [Blows, Addiction 2005] to 8.6 [Gjerde, AccAnalPrev 2011]); however, past hour self-reported exposure effectively doubled crash risk, even after controlling for confounding variables (adjusted point estimate OR, 1.84 [Asbridge, AccAnalPrev 2005] and 2.61 [Mann, JSafRes 2007]). Adjusted driver culpability ORs increased substantially when blood THC concentrations exceeded 5 ng/mL (OR 2.12 [Laumon, BMJ 2005] and 6.6 [Drummer, AccAnalPrev 2004]). Studies that employed urine as the analytical matrix did not show an association between cannabis and crash risk. Experimental: Sometimes drivers under the influence of cannabis attempted to compensate by driving more slowly; but their control deteriorated with increasing task complexity or divided attention requirements. Cannabis increased reaction time and lane position variability (standard deviation of lateral position, SDLP), and impaired cognitive function (critical tracking, planning). There was evidence for a tolerance effect in heavy (>4x weekly vs ≤1x/week) smokers on critical tracking and divided attention tasks. Tolerance to THC impairment was corroborated in cannabis-positive drivers apprehended for DUID, who were less likely to be judged 'impaired' with self-reported regular intake despite no difference in blood THC concentration. The proportion of observed impairments increased with serum THC concentrations. Combining cannabis with alcohol enhanced impairment, especially SDLP.

Conclusion: Different study designs or outcome measures frequently accounted for disagreement between results. Selection bias and confounding factors such as youth (≤35 years), sex, risky behaviors, and polydrug use altered or attenuated ostensible cannabis effects, but the association with MVAs retained significance in many studies. Evidence suggests recent smoking and/or blood THC concentrations ≥2 to 5 ng/mL (task dependent) are associated with significant driving impairment, particularly in occasional smokers. Urine is a suitable matrix for documenting cannabis exposure but not inferring driving impairment. Blood and oral fluid (OF) are thus preferable to urine for demonstrating recent intake, for onsite detection, logistical issues favor OF. Future cannabis driving research should emphasize challenging tasks, such as those based on divided attention, and include occasional as well as chronic daily cannabis smokers. Fully elucidating cannabis impact on driving skills is necessary for public safety.

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

Keywords: Cannabis, Driving Impairment, Marijuana, THC

S12

Sustained Abstinence Improves Psychomotor Function in Chronic Daily Cannabis Smokers

Wendy M. Bosker[1], Erin L. Karschner[2], Dayong Lee[2], Robert S. Goodwin[2], Jussi Hirvonen[3], Robert B. Innis[3], Eef L. Theunissen[1], Kim P. C. Kuypers[1], Johannes G. Ramaekers[1], **Marilyn A. Huestis*[2]**; [1]Dept. Neuropsychology & Psychopharmacology, Maastricht University, Maastricht, The Netherlands, [2]Chemistry & Drug Metabolism, IRP, National Institute on Drug Abuse, NIH, Baltimore, MD, USA, [3]Molecular Imaging Branch, National Institute of Mental Health, NIH, Bethesda, MD, USA

Background: Epidemiological studies document the impairing effects of cannabis on driving (Drummer et al 2004; Accident Anal Prev; Ramaekers et al 2000; Human Psychopharm). In laboratory studies, critical frequency (λ_c) of critical tracking, and tracking error and control losses on divided attention were shown to be sensitive to cannabis impairing effects. We hypothesized that psychomotor performance of chronic, daily cannabis smokers would be impaired on admission as compared to controls, and that performance would improve during sustained abstinence.

Objectives: To determine if psychomotor performance in non-intoxicated chronic daily cannabis smokers was impaired as compared to a control group of non-intoxicated occasional drug users; and whether psychomotor performance would improve following sustained cannabis abstinence.

Methods: Critical tracking measures the ability to control a displayed error signal in a 1st-order compensatory tracking task; with frequency of control losses λ_c or critical frequency as the primary dependent measure. Divided attention assesses the ability to divide attention between two tasks performed simultaneously, control losses and tracking errors are the primary outcome measures. Subjects received extensive training on psychomotor tests to assure stable performance. Psychomotor function of cannabis smokers was assessed at baseline and after 8, 14-16 and 21-23 days of continuously monitored abstinence on a closed research unit.

Results: Chronic, daily smokers (N=17) differed significantly from controls (N=30) ($F4;93=8.629$; $p<0.001$) at baseline ($p<0.001$), after 8 ($p=0.002$); 14-16 ($p=0.004$) and 21-23 days of abstinence ($p=0.008$) on critical tracking. Subjects performed better after 8 days ($F1;16=3.203$; $p=0.092$); after 14-16 days ($F1;16=3.487$; $p=0.080$) and after 21-23 days of abstinence ($F1;11=-5.096$; $p=0.045$) compared to baseline. Control losses during divided attention significantly decreased after 14-16 days of abstinence ($F1;17=4.611$; $p=0.046$), but trended at 21-23 days of abstinence ($F1;11=3.850$; $p=0.076$), relative to baseline. Compared to the control group, chronic, daily cannabis smokers had significantly more control losses ($F4;93=5.051$; $p=0.001$), at baseline ($p=0.014$), 8 ($p=0.029$), 14-16 ($p=0.017$) and 21-23 days of abstinence ($p=0.004$).

Conclusions: Psychomotor performance in the critical tracking and divided attention tasks in 19 male chronic, daily cannabis smokers was impaired at baseline relative to the comparison group. Sustained cannabis abstinence moderately improved critical tracking and divided attention performance in chronic; daily cannabis smokers, although impairment was still observable compared to controls after 3 weeks of abstinence. We reported significant reductions in CB-1 cannabinoid receptors in specific brain areas of these participants (Hirvonen et al; Molecular Psychiatry 2011) within two days of admission. Over the same period of improvement in psychomotor performance, significant increases in CB1 cannabinoid receptors were observed. Withdrawal might have affected performance of chronic smokers; but primarily during the 1st week of abstinence. Also, impairments in chronic cannabis smokers may result from residual THC concentrations in blood (Karschner et al 2010; Addiction); which remained present for up to 24 days after last use. Chronic daily cannabis smokers had impaired psychomotor function as compared to occasional drug users for at least three weeks of sustained cannabis abstinence. Funded by the Intramural Research Programs, National Institutes on Drug Abuse and Mental Health, National Institutes of Health.

Keywords: Cannabis, THC, Sustained Abstinence, Chronic Cannabis Smokers, Divided Attention, Psychomotor Control

S13
Zolpidem and Driving - Case Reviews from Colorado

Sarah Urfer*; ChemaTox Laboratory, Boulder, CO, USA

Introduction: Zolpidem is a commonly prescribed sedative-hypnotic sleeping aid. The United States Food and Drug Administration (FDA) medication guide for zolpidem warns that a person taking zolpidem may get out of bed and perform a complex activity while not fully awake with no memory of the activity the next day. Driving, eating, talking on the phone, and sex are listed as possible activities. Zolpidem severely impairs driving ability even at therapeutic levels. Driving under the influence of drugs (DUID) cases with zolpidem are becoming more frequent in cases tested by ChemaTox Laboratory and in 2011 zolpidem was the sixth most frequent drug detected in the labs DUID cases.

Objective: During a 32-month period from July 2009 until February 2012, a review of all zolpidem positive cases were evaluated at ChemaTox Laboratory. The objective of the review was to identify signs and symptoms of zolpidem impairment and evaluate whether the zolpidem levels correlated with observed impairment. The review focused on identifying the following: behaviors that appear in multiple reports, the most frequent multiple drug intoxication combinations with zolpidem, and the concentrations of zolpidem in both single and multiple drug intoxication cases. Selected cases are examined in detail to highlight specific traits.

Methods: In the 32-month period reviewed, 28 driving under the influence of drugs (DUID) cases were identified as positive for zolpidem. All testing was performed on blood samples. The samples were screened by enzyme-linked immunosorbent assay (ELISA). All confirmation testing was by gas chromatography – mass spectrometry (GC-MS) with a limit of detection (LOD) of 20 ng/mL and limit of quantitation (LOQ) of 50 ng/mL whole blood. Five cases were positive below the LOQ of 50 ng/mL and all five had additional drugs or alcohol present. In the 23 cases where zolpidem was quantitated the mean zolpidem concentration was 419 ng/mL, median 280 ng/mL and range of 82 ng/mL to 1400 ng/mL. In 10 cases zolpidem was the only drug detected. The mean concentration of zolpidem in these single drug intoxication cases was 357 ng/mL, median 265 ng/mL and range of 140 ng/mL to 770 ng/mL. The most frequent drug found in combination with zolpidem was alcohol (n=9) with blood alcohol concentrations ranging from 0.034 – 0.185 g ethyl alcohol/ 100 mL blood. Other drugs found in combination with zolpidem were tramadol (n=3), methadone (n=2), oxycodone (n=2), and once each hydrocodone, codeine, morphine, methamphetamine, diphenhydramine, and THC.

Results: When police reports were available, they were compared to see if any of the documented signs of impairment were the same. Some of the factors listed in multiple reports were: hitting multiple stationary objects such as signs and parked cars, subjects having difficulty keeping their eyes open, poor memory of recent events, difficulty following instructions and answering questions, and poor reaction time.

Conclusion / Discussion: Detection of drugs and alcohol in blood samples for DUI/D cases is a critical part of maintaining public safety on the roadways. Alcohol is the most commonly detected substance but as prescription drug use and abuse rises, it is important that laboratories look for other impairing substances. In this review, 28 zolpidem driving cases were evaluated. The zolpidem concentrations and subject behavior at the time of contact were compared to better understand the relationship between zolpidem and DUID.

Keywords: Zolpidem, Driving, Impairment

S14
Driving Under the Influence of Drugs Involving Phenazepam in Virginia

Curt E. Harper, Emily Lawrence, Jason Hudson; Virginia Department of Forensic Science, Richmond, VA, USA

Introduction: Phenazepam is a benzodiazepine derivative which has been used clinically to treat anxiety, insomnia, alcohol withdrawal, and epilepsy in Russia since the late 1970's. It is not available by prescription in the United States; however, it is readily available in powder or tablet form through various internet websites.

Objective: To investigate the prevalence of phenazepam in DUI/D cases in Virginia and characterize observations and behavior noted by officers.

Method: Whole blood samples from apprehended drivers suspected of driving under the influence of drugs from 2010-2011 were analyzed for phenazepam. Samples were screened by ELISA. Phenazepam was analyzed in blood by adding sodium carbonate buffer and extracting with 1-chlorobutane. Quantitation was performed by HPLC-DAD and confirmed by LCMS.

Results: We identified eight phenazepam positive cases over this time period. The majority of cases (88%) were from Central and Northern Virginia. 75% of arrestees were Caucasian males. 83% were between the ages of 20 and 28 years-old. 6/8 cases involved polydrug use and THC was the most common additional drug found (50%). The mean concentration of phenazepam was 0.22 mg/L +/- 0.14 (median 0.18 mg/L). The range was 0.10 - 0.52 mg/L.
Two cases are presented below:

Case History 1: A 22 year-old Caucasian male was pulled over for speeding. The suspect used the car for balance and stumbled to the sidewalk where standardized field sobriety tests (SFSTs) were administered. During the Walk and Turn (WAT) test the suspect stepped off line and missed heel to toe on steps 3-9. He was unable to perform the One Leg Stand (OLS). The officer noted slurred speech and dilated pupils. The suspect admitted to drinking one glass of wine and using “Jamaica Man". Toxicology revealed phenazepam at 0.52 mg/L and THC at 0.001 mg/L.

Case History 2: A 28 year-old Caucasian male was stopped for weaving on a four-lane highway and running off the road. The individual displayed slurred speech, droopy eyelids, and drowsiness. The subject displayed dilated pupils, lacked smooth pursuit, stepped off line several times during the WAT, and could not focus on the officer's finger during HGN. The OLS was discontinued because the officer “was afraid he would fall over." He admitted to “taking benzos" which he bought from an ex-girlfriend. Toxicology revealed phenazepam at 0.14 mg/L, diazepam at 0.04 mg/L, and nordiazepam at 0.07 mg/L.

Conclusion: We have noticed a significant increase in phenazepam DUI/D cases over the past year. Based on user reports, it appears that phenazepam is often used in conjunction with or as a component of marijuana, synthetic cannabinoids, or baths salts, but the individual may not know what they are taking. Officers noted dilated pupils, lack of smooth pursuit, HGN, slurred speech, difficulty following instructions, and poor balance and coordination. Apprehended drivers displayed poor driving, psychomotor impairment, and poor performance on SFSTs. Contribution from polydrug use should be considered. However, an emphasis should be placed on phenazepam testing in forensic toxicology laboratories due to the continued increase in DUI/D cases involving phenazepam in the United States.

Keywords: Phenazepam, DUID, Benzodiazepines

S15

Arizona DUID and Cannabis: Three Case Reports Covering Observed Driving Behaviors, D.R.E. Evaluations and Toxicological Results in Arizona Drivers

Chester Flaxmayer*, Criminalist, Phoenix, AZ, USA

Introduction: Arizona has a DUID Admin Per Se law. Arizona Revised Statute (ARS) 28-1381A(3) makes it unlawful for a person to drive or be in actual physical control of a vehicle while there is any drug as defined in section ARS 13-3401 or its metabolite in the person's body, which includes cannabis. As a result, Arizona has a relatively large number of DUID cases in which drivers are stopped, a DRE is attempted or performed, a blood sample obtained, and a cannabinoid or its metabolite detected, resulting in the charge of ARS 28-1381A(3). In some instances, the measured THC or THC metabolite is detected at very low concentrations.

Objective: Three DUID case reports will be discussed covering the observed driving including the reasonable suspicion for the stop, field performance including the probable cause for arrest, the subsequent DRE performance, and the subsequent laboratory analysis of the blood for THC and its metabolites. These cases will be discussed to compare the observed driving, blood THC and metabolite concentrations and the reported DRE observations.

Method: Three cases were compared in which Arizona drivers had been charged with violating ARS 28-1381A (3). A comparison of these three cases includes driving behavior, field performance and DRE data, as well as blood analysis showing THC or its metabolite concentrations.

Results: These three cases show little if any poor driving and the drivers' blood contains relatively low concentrations of cannabinoid or synthetic cannabinoid metabolites. However, the DRE indicates the presence of numerous signs and symptoms of use, as well as articulable impairment.

Conclusion: An Admin Per Se statute enables law enforcement to detain, arrest, examine, and draw blood from drivers stopped for suspicion of driving under the influence of drugs in Arizona. These cases show some of the issues that arise from such a law and the types of driving behavior, field and DRE performance, and blood concentrations of THC or its metabolite found in the blood of Arizona drivers.

Keywords: DUID, Driving, Cannabinoid, D.R.E., Blood, THC

S16
Detecting and Distinguishing 5-MeO-DIPT from Ropinirole in Wisconsin Drivers

William Johnson*, Amy Miles; Wisconsin State Laboratory of Hygiene, Toxicology Section, Madison, WI, USA

Introduction: 5-methoxy-N,N-diisopropyltryptamine (5-MeO-DIPT, Foxy, Foxy Methoxy) is a tryptamine derivative, structurally similar to psilocybin and psilocin, used for its hallucinogenic effects. Acting as a 5-HT2 agonist, primary effects include dilated pupils, euphoria, hallucinations, visual and audio distortions, emotional distress, nausea and vomiting. Psychedelic effects occur 20 - 30 minutes following a 6 - 20 mg oral dose. Peak effects following oral administration occur within 1 - 1.5 hours of use while duration of effects last from 3 - 6 hours, yielding an overall experience similar to LSD or psilocybin. Use via insufflation and smoking has been reported, but users note these routes require a larger dose to achieve the same effect as oral dosing. Users also report marijuana mitigates the more intense visual and audio distortions of 5-MeO-DIPT. The compound was classified as Schedule I under the United States Controlled Substance Act in 2004 and is currently listed in Wisconsin's Restricted Controlled Substance (RCS) statute.

Objective: Structural similarities and nearly identical mass spectra shared between 5-MeO-DIPT and ropinirole (Requip) pose an analytical challenge and potential risk for misidentification. 5-MeO-DIPT was first flagged as an unknown at the Wisconsin State Laboratory of Hygiene (WSLH) in an Operating While Intoxicated (OWI) blood specimen in July 2011. Further investigation narrowed the identification to either 5-MeO-DIPT or ropinirole, commonly used for Parkinson's disease and Restless Leg Syndrome.

Method: Analytical standards for each were evaluated utilizing an n-butyl chloride liquid:liquid extraction with acid back extraction. Analysis was performed by gas chromatography with nitrogen phosphorus and mass selective detection (GC/MS).

Results: While both mass spectra were very similar to the unknown and contained the distinctive 114 amu base peak, 5-MeO-DIPT was the clear match. Secondary ions noted were 160, 174, 145, 130 for 5-MeO-DIPT and 160, 231, and 132 for ropinirole. For reference, the elution order of these and related compounds was: imipramine, ropinirole, 5-MeO-DIPT, cyclobenzaprine. Separate quantitations of 5-MeO-DIPT were not performed as it is reported “present" under Wisconsin's RCS statute.

Age	Sex	BAC (g/dL)	5-MeO-DIPT (ng/mL)	Other Drugs found
28	F	0.088	~155	THC
28	F	0.076	~165	diph, sert, norsert, BE, morphine, levam
24	F	0.064	~70	THC
17	M	ND	~70	THC
21	M	0.032	~45	THC

Conclusion/Discussion: Extraction of 5-MeO-DIPT and ropinirole standards within the same run followed by careful evaluation of their GC/MS spectra was critical in distinguishing between both compounds. Confidence in this identification is crucial in reporting 5-MeO-DIPT without additional quantitation. The combination of 5-MeO-DIPT and cannabinoids in four cases could be intentional use as noted in Erowid testimonials or unsuspecting use of adulterated marijuana.

Keywords: 5-MeO-DIPT, Hallucinogen, Ropinirole, Drivers

S17

Cannabinoid Stability in Authentic Oral Fluid Collected by Expectoration and with the Quantisal™ Device After Controlled Cannabis Smoking

Dayong Lee*, Garry Milman, David M. Schwope, Allan J. Barnes, David A. Gorelick, Marilyn A. Huestis; Chemistry and Drug Metabolism, IRP, National Institute on Drug Abuse, NIH, Baltimore, MD, USA

Introduction: Defining oral fluid (OF) drug stability is an important consideration for OF result interpretation. There are few OF stability data, and none based on authentic OF following controlled cannabis smoking. We evaluated the stability of Δ^9-tetrahydrocannabinol (THC), 11-nor-9-carboxy-THC (THCCOOH), cannabidiol (CBD), and cannabinol (CBN) in authentic OF stored at 4°C for 1 and 4 wks, and at -20°C for 4 and 24 wks.

Methods: Healthy 18-45 year old cannabis smokers provided written informed consent for this Institutional Review Board-approved study. An expectorated OF pool and a pool of OF collected with Quantisal™ devices were prepared for each participant following smoking of a 6.8% THC cigarette. Cannabinoids were quantified by 2D-GC/MS. Limits of quantification were 0.5 ng/mL (THC and CBD), 1 ng/mL (CBN), and 7.5 pg/mL (THCCOOH) in Quantisal specimens, and 0.25 ng/mL (THC and CBD), 1 ng/mL (CBN), and 5 pg/mL (THCCOOH) in expectorated specimens. Results within ±20% of baseline concentrations analyzed within 24 h were considered stable.

Results: Of 40 data points per analyte from 10 participants, % baseline concentrations were determined for 40 THC, 36 THCCOOH, 33 CBD, and 37 CBN Quantisal specimens and for 40 THC, 33 THCCOOH, 30 CBD, and 24 CBN expectorated specimens; results could not be determined for some pools due to analytical issues. All THC, THCCOOH, CBD and CBN concentrations were stable for 1 wk at 4°C. After 4 wks at 4°C, and 4 and 24 wks at -20°C, THC was stable in 90, 80 and 80%, and THCCOOH in 89, 40 and 50% of Quantisal specimens, respectively. Cannabinoids in expectorated OF were less stable than in Quantisal specimens whether refrigerated or frozen (Table); ≤67% of expectorated specimens were stable for THC and THCCOOH under 4 storage conditions. After 4 wks at 4 and -20°C, CBD and CBN were stable in 33-100% of Quantisal and expectorated samples; by 24 wks at -20°C, CBD and CBN were stable in ≤44%.

Conclusions: Cannabinoid OF stability varied by analyte, collection method, storage duration and temperature with large inter-subject variability. Overall, cannabinoids in OF collected with the Quantisal device were more stable and had more consistent quantifications than cannabinoids in expectorated OF. OF collection with a device containing a stabilization buffer, sample storage at 4°C and analysis within 4 wks is preferred to maximize result accuracy.

Supported by the Intramural Research Program, National Institute on Drug Abuse, NIH

Keywords: Oral Fluid, Tetrahydrocannabinol, Cannabinoids, Stability

S18

In Vitro Stability of Cannabinoids and Cannabinoid Glucuronides in Authentic Whole Blood and Plasma Following Controlled Smoked Cannabis

Nathalie A. Desrosiers*, Karl B. Scheidweiler, David M. Schwope, Erin L. Karschner, David A. Gorelick, Marilyn A. Huestis; Chemistry & Drug Metabolism, IRP, NIDA, NIH, Baltimore, MD, USA

Introduction: Analyte stability is critical for interpreting cannabinoid concentrations in clinical, forensic, and driving cases. Few data are available on the stability of cannabinoids in authentic cannabinoid whole blood (WB) and plasma specimens.

Objective: We examined free and glucuronide-conjugated cannabinoid stability in authentic specimens stored at 4 and -20°C for up to 12 months following controlled smoked cannabis.

Methods: 10 chronic daily cannabis smokers (9M, 1F) provided written informed consent to participate in this IRB-approved study. WB and plasma cannabinoids were quantified by LCMSMS after ad libitum smoking of one 6.8% THC cigarette. Low and high concentration pools were prepared for each participant within 2 h of collection by mixing specimens collected at 2, 3, and 4 h (low) and at 0.25, 0.5 and 1 h (high) post-smoking. Baseline specimens were analyzed within 24 h. Specimens were stored in polypropylene cryotubes at 4 or -20ºC and analyzed after 7, 14, 30, 90, and 180 days, and after 365 days for frozen specimens only. Limits of quantification (LOQ) in plasma and WB were 1, 1, 0.5 and 5 ng/mL for Δ^9-tetrahydrocannabinol (THC), 11-nor-9-carboxy-THC (THCCOOH), THC-glucuronide and THCCOOH-glucuronide, respectively. Specimens were considered stable if concentrations quantified within ±20% of baseline.

Results: THC, THCCOOH and THCCOOH-glucuronide baseline concentrations were >LOQ in all specimens; THC-glucuronide concentrations were >LOQ in 1 and 6 baseline high WB and plasma pools, respectively. THC WB concentrations were stable at 4 and -20°C through 90 days; however, mean THC loss in WB was >44% after 180 days at 4 and -20°C (>80% loss after 365 days at -20°C). THCCOOH WB concentrations were stable for at least 4 weeks refrigerated and one year frozen. Mean WB THCCOOH-glucuronide concentrations decreased >20% after 30 and >89% after 180 days storage at 4°C; at -20°C, decreases were >32% after 180 and >40% after 365 days. Plasma THC-glucuronide and THC concentrations were stable at 4 and -20°C throughout the study. THCCOOH increases of >148% were observed after 180 days at 4°C in plasma but was stable throughout the study at -20°C. Plasma THCCOOH-glucuronide loss exceeded 20% after 7 days at 4°C for 3 and 6 participants in low and high pools, respectively, with mean losses of >95 and >33% after 180 days at 4 and -20°C, respectively (>29% after 365 days at -20°C). Discussion: These are the most extensive cannabinoid stability studies to date in authentic WB and plasma specimens following controlled cannabis smoking and will be useful for interpreting cannabinoid results. Frozen specimen storage provided maximum stability, although <30 day refrigerated storage may be acceptable for WB determinations where THCCOOH-glucuronide analysis is not required. Refrigerated plasma storage is not recommended for >7 days when free THCCOOH concentrations are required, due to deconjugation of THCCOOH-glucuronide. Substantial THC loss in WB was observed beyond 90 days storage at 4 and -20°C, potentially confounding impairment interpretation. Possible explanations for the differences between matrices are differential protein binding, adsorption to containers or other unknown mechanisms.

Keywords: Cannabinoids, Whole Blood, Plasma, Stability

S19

Dose-Dependent Plasma Cannabinoid Concentrations after Controlled Oral Synthetic THC and Smoked Cannabis Administration in Chronic Daily Smokers

Garry Milman*[1], Mateus M. Bergamaschi[1], Dayong Lee[1], Damodara R. Mendu[1], Allan J. Barnes[1], Ryan Vandrey[2], and Marilyn A. Huestis[1]; [1]Chemistry and Drug Metabolism, Intramural Research Program, National Institute on Drug Abuse, NIH, Baltimore, MD, USA, [2]Behavioral Pharmacology Research Unit, Johns Hopkins Bayview Medical Center, Baltimore, MD, USA

Introduction: Dronabinol, oral synthetic delta-9-tetrahydrocannabinol (THC), is an approved medication for nausea/vomiting following chemotherapy and HIV-related wasting disease. An investigation of dronabinol's suppression of cannabis withdrawal provided the first data of which we are aware from chronic daily cannabis smokers that evaluates pharmacokinetics of smoked cannabis in individuals also receiving oral dronabinol.

Methods: 11 daily cannabis smokers provided written informed consent to participate in this IRB-approved study and resided on a secure research unit for 51 days. After 4 days of ad-libitum cannabis smoking, participants had four 5-day periods of smoked cannabis abstinence (during which they received 0, 10, 20, or 40-mg dronabinol 3 times/day), each separated by a 9-day period of ad-libitum cannabis smoking. At 1130 on the 5th day of each cannabis abstinence period, participants smoked one cannabis cigarette (5.9% THC). 56 plasma specimens were collected: 4 (0900, 1400, 1900, 2200) on the 1st day of each abstinence period and 10 (0900, 1100, 1145, 1230, 1400, 1530, 1700, 1900, 2030, 2200) on the 5th day, prior to and after cannabis smoking. Plasma THC, 11-hydroxy-THC (11-OH-THC), and 11-nor-9-carboxy-THC (THCCOOH) concentrations were quantified by two-dimensional GCMS. Linear ranges (ng/mL) were 0.5-100 for THC, 1.0-50 11-OH-THC, and 0.5-200 THCCOOH.

Results: Median baseline concentrations at the start of each abstinence period were similar between dose groups of 10, 20, and 40-mg dronabinol (THC 7.8-9.8 ng/mL, 11-OH-THC 3.7-4.8 ng/mL, and THCCOOH 93.5-125 ng/mL). Median cannabinoid concentrations at the start (0900) of the 5th abstinence day (14h after the previous dronabinol dose) were not significantly different from the baseline concentrations for THC (3.9-5.0 ng/mL) and 11-OH-THC (2.0-4.9 ng/mL), but significantly higher and dose-related for THCCOOH (73.6-189 ng/mL) for 10, 20, and 40-mg dronabinol smoked cannabis abstinence periods. Median maximal concentrations occurred 0.25h after smoking for THC (42.5, 31.2, 33.9 ng/mL, respectively) and 11-OH-THC (9.2, 9.9, 15.1 ng/mL, respectively). Median THCCOOH concentrations were highest 0.25 h after smoking for the 10 (122 ng/mL) and 20-mg dose (178 ng/mL); however, maximal THCCOOH (240 ng/mL) occurred at -0.5h prior to smoking for the 40-mg dose. Median concentrations of all cannabinoids, at all doses, did not increase significantly after the 14th (2.5h after smoking) and 15th (7.5h after smoking) oral dronabinol dose.

Conclusions: Plasma THC, 11-OH-THC, and THCCOOH concentrations increased dose-dependently over the first day of each abstinence period. Only psychoactive cannabinoids decreased to baseline after 14h of abstinence, demonstrating a lack of accumulation in plasma, possibly due to induced gastrointestinal metabolism and distribution into adipose tissue. THC maximum concentrations after smoking cannabis were much higher than after oral dronabinol, and were not correlated with dronabinol dose. 11-OH-THC concentrations were 1.5-2.5 times higher after 40-mg dronabinol than after 10 mg or 20 mg. THCCOOH concentrations accumulated after repetitive oral dronabinol in a dose-dependent manner and did not increase significantly after cannabis smoking.

Supported by NIDA-IRP, NIH and NIDA-DA025044.

Keywords: Plasma, Dronabinol, Cannabinoids, Smoked Cannabis

S20

Cannabinoid Plasma Concentrations in Chronic Daily Cannabis Smokers During Ad Libitum Smoking

Mateus M. Bergamaschi*[1,2], Garry Milman[1], Dayong Lee[1], Damodara R. Mendu[1], Allan Barnes[1], Regina H. C. Queiroz[2], Ryan Vandrey[3], Marilyn A. Huestis[1]; [1]Chemistry and Drug Metabolism, IRP, National Institute on Drug Abuse, NIH, Baltimore, MD, USA, [2]School of Pharmaceutical Science, University of São Paulo, Ribeirão Preto, SP, Brazil, [3]Department of Psychiatry and Behavioral Sciences, John Hopkins Bayview Medical Center, Baltimore, MD, USA

Introduction: More individuals in the U.S. are dependent on cannabis than on any other illicit drug. Δ9-tetrahydrocannabinol (THC), the primary psychoactive constituent of cannabis, is metabolized by cytochrome P450 2C9 and 2C19 to equipotent 11-hydroxy-THC (11-OH-THC), and further oxidized to 11-nor-9-carboxy-THC (THCCOOH).

Objective: Evaluate cannabinoid pharmacokinetics in chronic daily cannabis smokers participating in ad libitum smoking of multiple cannabis cigarettes.

Methods: Cannabis smokers provided written informed consent to participate in this John Hopkins Bayview Medical Center IRB-approved study. Participants resided on a closed research unit with unlimited access to cannabis cigarettes (5.9% THC) from noon to 11 pm. Limits of quantification (LOQ) were 0.5 ng/mL for THC and THCCOOH and 1.0 ng/mL for 11-OH-THC and cannabidiol (CBD).

Results: 7 volunteers smoked 174 THC cigarettes during two ad libitum smoking sessions separated by a 5-day abstinence phase. Ten plasma specimens were collected while participants smoked 1—26 joints/day; 4 additional specimens were collected the following day from 9 am to 10 pm during abstinence, yielding a total of 166 specimens (2 missing). Plasma specimens collected during ad libitum smoking and the abstinence phase were grouped by time from last cigarette smoked. Median plasma concentrations in 6 of 7 participants on admission were 3.8 (THC, range <LOQ—13.9), 0.6 (11-OH-THC, <LOQ—9.9) and 28.9 (THCCOOH, 3.3—242) ng/mL, with detection rates of 83% (THC), 50% (11-OH-THC), and 100% (THCCOOH). 0.5 h prior to the first smoked cannabis cigarette on ad libitum smoking days, median (n=7) cannabinoid concentrations were 2.7 (THC, 0.8—13.7), 1.4 (11-OH-THC, <LOQ—6.8) and 40.4 (THCCOOH, 20.1—226) ng/mL. The highest median concentrations, 41.6 (THC, 6.7—211), 7.3 (11-OH-THC, 2.7—41.3) and 68.0 (THCCOOH, 30.4—288) ng/mL occurred within 0.1-0.5 h after cannabis smoking. Median cannabinoid concentrations at 10 pm on smoking days were 13.1 (THC, 3.0—16.9), 4.0 (11-OH-THC, 2.1—9.9), and 59.0 (THCCOOH, 31.0—207) ng/mL, with all cannabinoids >LOQ. After 11 h of abstinence, median concentrations decreased to 2.9 (THC, 1.0—10.2), 1.8 (11-OH-THC, <LOQ—7.0), and 53.8 (THCCOOH, 32.4—348) ng/mL, with 2 specimens negative for 11-OH-THC. After 24 h of abstinence, median plasma concentrations in 6 of 7 participants were 4.4 (THC, 0.8—9.9), 1.6 (11-OH-THC, <LOQ—2.9, 2 negatives) and 49.9 (THCCOOH, 24.5—175) ng/mL; blood was not collected from one participant. CBD was not detected in any specimen; cannabis cigarettes contained only 0.01% CBD.

Conclusion: These data provide novel information about cannabinoid excretion profiles in chronic daily cannabis smokers during and after multiple smoked cannabis cigarettes. We documented extended plasma elimination, with positive THC and THCCOOH concentrations after 20-27 h of abstinence. THC and 11-OH-THC returned to admission plasma concentrations at the end of the 5-day abstinence phase, while THCCOOH concentration remained elevated. The increased cannabinoid body burden after chronic daily cannabis smoking results in an extended detection window of detection in plasma for THC, 11-OH-THC, and THCCOOH.

Supported by the National Institutes of Health, Intramural Research Program, NIDA and FAPESP (São Paulo Research Foundation, Brazil).

Keywords: Cannabinoid, Δ9-tetrahydrocannabinol, Window of Detection

S21

Ultra-Sensitive Detection of 11-Nor-delta-9-Tetrahydrocannabinol-9-Carboxylic Acid and Simultaneous Detection of Delta-9-Tetrahydrocannabinol in Oral Fluid by Microflow LC-MS/MS Using Benchtop Quadrupole/Orbitrap Mass Spectrometer

Xiang He*, Marta Kozak, Subodh Nimkar; ThermoFisher Scientific, San Jose, CA, USA

Background: Delta-9-Tetrahydrocannabinol (THC) has been used for detection of cannabis in oral fluid. THC-carboxylic acid (THCA), a metabolite of THC, has also been used for cannabis detection because to eliminate possibility of passive exposure. However, mainly due to the very low concentration of THCA (10-200 pg/mL) in oral fluid, such test can only be done with gas chromatography tandem mass spectrometry (GC-MS/MS) or 2-dimensional GC-MS. Because of the high complexity in sample preparation and instrument setup, such technique has not been widely adopted.

Objective: To develop a simple and sensitive quantitative liquid chromatography tandem mass spectrometry (LC-MS/MS) method for THCA in oral fluid with microflow chromatography and high resolution accurate mass (HRAM) quadrupole/Orbitrap mass spectrometer.

Methods: Oral fluid samples were diluted with preservation buffer in collection device and spiked with internal standard (THCA-d9). The mixture was directly injected for microflow-based LC-MS/MS analysis with minimal pre-treatment and online clean-up. The LC-MS/MS analysis was performed on a Dionex Ultimate 3000 RSLCnano system operated in microflow mode coupled to a Thermo Scientific Q Exactive mass spectrometer with heated electrospray ionization (HESI-II) source operated in negative ion mode. Targeted-MS2 scan mode was used for data acquisition. In this scan mode, 313.2 (THC), 316.2 (THC-d3), 343.2 (THCA) and 352.2 (THCA-d9) were isolated in the quadrupole with 3 *m/z* window and subsequently fragmented in Higher Energy Collisional Dissociation cell (HCD), and with resolution of 70,000 (FWHM at *m/z* 200). Major fragmented ions from THC and THCA were extracted with 5 ppm mass accuracy and used for quantitation and confirmation. LC with online sample clean-up was carried out on a Hypersil GOLD aQ pre-concentration column (10 ×1 mm; 3 µm particle size) and microflow LC column (100 × 0.32 mm; 3 µm particle size) at 35 degrees celsius.

Results:

1. Oral fluid samples collected in commercially available collection kit with preservation buffer were directly injected for LC-MS/MS analysis. Liquid-liquid extraction and solid phase extraction were tested and their performances were compared to the direct injection approach.
2. The method is 12.5 minutes long. Based on preliminary validation results in synthetic oral fluid samples, the method has a lower limit of quantitation of 7.5 pg/mL and is linear from 7.5 to 300 pg/mL. The intra and inter-batch precision of the method for THCA ranged from 3.7 to 12.5% for the two quality control samples at 24 and 120 pg/mL. Real oral fluid samples were spiked with THCA and THC and tested for method robustness and matrix effects. Oral fluid samples tested as THC-positive are being acquired and will be presented in the final presentation.

Results from the full validation will be reported in the final presentation.

Conclusion: We have successfully developed a simple, robust and highly sensitive microflow LC-MS/MS method for the quantitation of THCA and simultaneous detection of THC in oral fluid using Q Exactive Mass Spectrometer. To our knowledge, this is the first report of LC-MS/MS-based quantitative analysis of THCA in oral fluid with minimum sample preparation and without the need for derivatization.

Keywords: THCA, Oral Fluid, LC-MS/MS, Microflow, Q Exactive

S22

UPLC-TOF MS Discrimination of a Norbuprenorphine Glucuronide Interference Observed in UPLC/MS/MS Urinalysis

Ayodele Collins*[1], Gregory L. McIntire[2], Ronald C. Backer[1]; [1]Ameritox, Ltd., Midland, TX, USA, [2]Ameritox, Ltd., Greensboro, NC, USA

Introduction: Buprenorphine is a synthetic opiate analgesic commonly prescribed for pain management. It is metabolized in man primarily by N-dealkylation and conjugation. For compliance testing, the advantages of excluding sample hydrolysis and monitoring buprenorphine, norbuprenorphine and the corresponding glucuronides using Ultra Performance Liquid Chromatography Tandem Mass Spectrometry (UPLC/MS/MS) have been reported. Recently a number of specimens that underwent direct UPLC/MS/MS confirmation testing were identified as having only norbuprenorphine glucuronide, an unusual metabolic pattern. An investigation of these specimens using a Time of Flight Mass Spectrometer (TOF MS) demonstrated by exact mass that in spite of meeting all criteria for retention time and chosen ion transitions on a triple quadrupole detector (TQD). The substance identified was not norbuprenorphine glucuronide. The identification of the interference is still under investigation. An alternative protocol involving enzyme hydrolysis was also developed to distinguish the apparent pseudo-compound. Under enzyme hydrolysis, true norbuprenorphine glucuronide would yield norbuprenorphine, which can be confirmed by UPLC/MS/MS with no known interference. Limitations of LC/MS/MS without exact mass identification and possible resolutions are highlighted in this examination.

Methods: Urine samples submitted for buprenorphine determinations were analyzed (unhydrolyzed) after dilution on a Waters Acquity UPLC TQD. Samples showing the unusual metabolic pattern of only norbuprenorphine glucuronide (n=9) were reanalyzed using a Waters Acquity UPLC Xevo G2 TOF MS (resolution 20;000 fwhm, leucine enkephalin lock mass, low and elevated energy mode). Accurate mass measurement for precursor and fragment ions, fragmentation pattern, retention time and predicted elemental composition relative to a norbuprenorphine glucuronide reference standard were assessed on the TOF. Several samples along with a norbuprenorphine glucuronide control were then hydrolyzed with glucuronidase and reanalyzed by UPLC/MS/MS. Analyses on the TOF and TQD were completed in positive electrospray ionization mode.

Results: The accurate mass measurement of the norbuprenorphine glucuronide precursor ion was 590.2965 (mass error= 0.0ppm); which generated a consistent best match formula of C31H44NO10 for elemental composition. Fragment ions in the associated TOF spectrum were 414.2615; 396.2506; and 101.0940. For all discrepant patient specimens, an exact mass ranging between 590.2696 and 590.2728 was observed at the norbuprenorphine glucuronide retention time. The mass difference from the norbuprenorphine glucuronide exact mass exceeded 26.9 mDa. Predicted elemental compositions did not match that of norbuprenorphine glucuronide. Fragments ions were similar in nominal mass, but exact mass differences exceeded 23.9 mDa and relative intensities were inconsistent. This indicated that the identified compound was not norbuprenorphine glucuronide. The enzyme hydrolyzed specimens containing the pseudo-norbuprenorphine glucuronide did not show norbuprenorphine upon UPLC/MS/MS reanalysis, while the hydrolyzed control showed the expected presence of norbuprenorphine. Furthermore, enzyme hydrolysis did not eliminate the interferent peak.

Discussion: Buprenorphine confirmation results for unhydrolyzed specimens with unusual metabolic patterns must be interpreted with caution. Such occurrences can be further examined with a TOF exact mass instrument. Alternatively, the specimens can be hydrolyzed and analyzed for the expected free analyte. An enzyme immunoassay that is cross-reactive with the glucuronide or the unconjugated analyte may also be employed for preliminary testing, providing a secondary level of confirmation.

Keywords: Norbuprenorphine Glucuronide, Interference, UPLC-TOF MS

S23

Prediction of Drug Interactions with Methadone, Buprenorphine and Oxycodone From In Vitro Inhibition of Metabolism: Study Design

David E. Moody*, Wenfang B. Fang; Center for Human Toxicology, University of Utah, Salt Lake City, UT, USA

Introduction: The Research, Development and Evaluation committee of the Special Working Group on Forensic Toxicology (SWGTOX) designated important areas for future research. Potential for drug interactions was one of these. Opioid mortality is a growing concern and drug interactions have been recognized as a contributing factor.

Objectives: To design a study that may predict potential drug interactions from in vitro inhibition of opioid metabolism. Methadone, oxycodone and buprenorphine were chosen as candidate opioids as LC-MSMS methods for metabolic pathways of these drugs have already been developed in our laboratory. These include: R- and S-methadone to R- and S-EDDP; oxycodone to noroxycodone and oxymorphine; and buprenorphine to norbuprenorphine; buprenorphine-3-glucuronide and norbuprenorphine-3-glucuronide.

Methods: A list of 102 drugs were gathered from: H_2-receptor antagonists; proton pump inhibitors; imidazole antifungals; macrolid antibiotics; calcium channel blockers; ß-blockers; tricyclic antidepressants; SSRIs; SNRIs; antipsychotics and benzodiazepines as these groups have been associated with a number of drug interactions. First a two-set three-concentration screen would be conducted in human liver microsomes (HLM). In one set, the inhibitor would be added along with drug and co-factors for cytochrome P450 (CYP) (and for buprenorphine UDP-glucuronosyl-transferase (UGT). In another, the inhibitor would be preincubated with HLM and co-factors to test for metabolism-dependent inhibition. For compounds that demonstrated inhibition of metabolism, more thorough studies would be conducted to determine an IC_{50} for reversible or K_i and k_{inact} for metabolism-dependent inhibitors. These incubations would use cDNA-expressed CYPs and UGTs involved in the metabolism of each opioid.

Results: The three-year proposal was found acceptable and funded (effective 1/17.12) by the National Institute of Justice. Funding included travel to annual SOFT meetings to present findings to the forensic toxicology community.

Conclusions: While this abstract only addresses study design, we hope this by itself is an important aspect to report to our forensic toxicology community. We anticipate adding preliminary data by the time of the meeting.

Funding: This project was supported by Award No. 2011-DN-BX-K532; awarded by the National Institute of Justice; Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect those of the Department of Justice.

Keywords: Buprenorphine, Methadone, Oxycodone, In Vitro Metabolism, Potential Drug Interactions

S24

Lefetamine-Derived Designer Drugs: Which Role Do the Human Hepatic Cytochrome P450 Isoenzymes Play in the Formation of Metabolites of N-ethyl-1,2-diphenylethylamine (NEDPA) and N-iso-propyl-1,2-diphenylethylamine (NPDPA)?

Carina S. D. Wink, Markus R. Meyer, Hans H. Maurer; Department of Experimental and Clinical Toxicology, Saarland University, Homburg (Saar), Germany

Introduction: Lefetamine (SPA, N,N-dimethyl-1,2-diphenylethylamine) was marketed as a central acting opioid (Santenol®). After frequent misuse, it was scheduled in Germany, but none of its derivatives. In 2008, the two lefetamine-derivatives NEDPA and NPDPA were confiscated in a clandestine drug laboratory in Germany. Former studies showed that NEDPA and NPDPA were mainly N-dealkylated and hydroxylated at the benzyl moiety in rat (CSD Wink et al., IATDMCT congress, 2011).

Objective: The aim of the presented work was to study the involvement of CYP isoenzymes in the initial N-dealkylation of NEDPA and NPDPA.

Methods: Baculovirus infected insect cell microsomes with cDNA-expressed human P450 (1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4 and 3A5) and pooled human liver microsomes (pHLM) were tested in an initial activity screening. 1,2-Diphenylethylamine, the common N-dealkyl metabolite, was analyzed by LC-HR-MS (TF Exactive). For the isoenzymes involved, Km and Vmax were determined. Using the relative activity factor (RAF) approach, the hepatic clearance was calculated. Incubation, LC-MS analysis, and RAF approach were performed according to MR Meyer et al., JMS, 2012.

Results: In the initial activity screening, only CYP 1A2, 2B6, 2C19, 2D6, and 3A4 were capable of catalyzing the N-dealkylation of NEDPA and NPDPA. They showed classic Michaelis-Menten kinetics for NEDPA N-dealkylation in the expected plasma concentration range with Km values (µM) of 238 (CYP 1A2), 122 (2B6), 2 (2C19), 1 (2D6), 263 (3A4), and 144 (pHLM) and Vmax values (pmol/min/pmol) of 4 (CYP 1A2), 6 (2B6), 0.4 (2C19), 0.25 (2D6), and 7 (3A4) for the isoenzymes, and 58 pmol/min/mg for pHLM. For NPDPA N-dealkylation, the Km and Vmax values were as follows: Km (µM): 181 (CYP 1A2), 72 (2B6), 6 (2C19), 0.75 (2D6), 251 (3A4), 104 (pHLM), Vmax (pmol/min/pmol): 0.6 (1A2), 1.7 (2B6), 1.3 (2C19), 0.03 (2D6), 3 (3A4), and 79 pmol/min/mg (HLM). Using the RAF approach, the hepatic clearance was calculated to be 57% for the N-dealkylation of NEDPA by CYP3A4 and 25% by 2B6, less than 8% each by CYP1A2, 2C19 and 2D6 and 61% for the N-dealkylation of NPDPA by CYP3A4 and 24% by 2B6, also less than 8% each by CYP1A2, 2C19 and 2D6.

Conclusion: NEDPA and NPDPA were both N-dealkylated by CYP 1A2, 2B6, 2C19, 2D6, and 3A4. Because of the low amount of CYP2C19 in HLM compared to the cDNA-expressed enzyme and their low affinity to CYP2D6, the N-dealkylation in vivo should mainly be catalyzed by CYP2B6 and 3A4. Thus, interactions must be considered with their inhibitors or inducers as well as with drugs, food or xenobiotics also metabolized by these CYPs.

Keywords: Designer Drugs, NEDPA, NPDPA, CYP, LC-HR-MS

S25

Application of the Substrate Depletion Approach for Investigation of Enantioselective Sulfation of 3,4-methylenedioxyethylamphetamine (MDEA)'s Main Metabolites

Andrea E Schwaninger*[1,2], Markus R. Meyer[1], Hans H. Maurer[1]; [1]Department of Experimental and Clinical Toxicology, Saarland University, Homburg, Germany, [2]Department of Forensic Pharmacology and Toxicology, Institute of Forensic Medicine, University of Zurich, Zurich, Switzerland

Background: The two enantiomers of the 3,4-methylendioxymethamphetamine (MDMA)-derived designer drug 3,4-methylenedioxyethylamphetamine (MDEA) showed different excretion kinetics in vivo. For MDMA, estimation of ingestion time was possible via their R/S ratios in human urine, with best results for the phase II metabolites. To assess whether similar calculations can be applied to MDEA, initial in vitro experiments on the stereoselectivity of MDEA's phase II metabolism are needed.

Objectives: As chiral separation of 3,4-dihydroxyethylamphetmine (DHEA) sulfate and 4-hydroxy-3-methoxyethylamphetamine (HMEA) sulfate could not be achieved; monitoring of substrate depletion was evaluated as an alternative approach to study enantioselective sulfation in human liver cytosol (HLC) and recombinant sulfotransferases (rSULT)s in vitro. Sulfation should be of major importance, especially considering the detoxification of DHEA, the reactive dihydroxy metabolite of MDEA

Methods: Achiral kinetic data for DHEA and HMEA sulfation were derived from incubations in HLC or rSULT with substrate concentrations of 0.05 - 500 µM monitoring product formation by LC-HRMS (TF Exactive; approach 1). For enantioselective kinetic data, substrate loss was determined over time (0, 5, 10, 15, 20 min) at eight substrate concentrations after chiral derivatization with S-heptafluorobutylprolylchloride and GC-NICI-MS analysis (AT MSD; approach 2). From percentages of remaining substrate, initial substrate depletion rates (slope of the natural log of the percentage remaining over time) were calculated and used for Km and Vmax assessment (Obach; Drug Metabol Disp; 2002). Applicability of the substrate depletion approach was checked by comparison of Km values obtained for the two approaches.

Results: From the five major SULT isoenzymes tested, SULT1A3 was the main enzyme catalyzing DHEA and HMEA sulfation. Km values calculated by a chiral product formation (approach 1) for sulfation of DHEA and HMEA in HLC were 3.8 +/- 1.3 µM and 0.67 +/- 0.11 µM; and by recombinant SULT1A3 3.1 +/- 0.8 µM and 0.51 +/- 0.01 µM; respectively. The kinetic data showed deviation from typical Michaelis-Menten kinetics in terms of substrate inhibition. Assessment via substrate depletion rates (approach 2) resulted in the following Km values for SULT1A3: 0.4 +/- 0.1 µM (R-DHEA); 0.8 +/- 0.2 µM (S-DHEA); 0.3 +/- 0.06 µM (R-HMEA); and 0.2 +/- 0.06 µM (S-HMEA). Good correlation between the two approaches was observed for HMEA and still acceptable correlation for DHEA with values between twofold of each other. Vmax values could be obtained from substrate depletion experiments and showed large differences between the enantiomers. DHEA sulfation activity was about 2-fold higher for the S-enantiomer, whereas the opposite was observed for HMEA.

Conclusion: In conclusion, sulfation of DHEA and HMEA was mainly catalyzed by SULT1A3 and also revealed different enantiomeric disposition. DHEA showed, as O-demethylation, methylation and glucuronidation, preferences for the S-enantiomer whereas higher efficiencies were observed for R-HMEA sulfation. That is in contrast to MDMA where sulfation of the hydroxy-methoxy metabolite was not enantioselective. Generally, the substrate depletion approach was suitable for determination of chiral sulfation kinetics and showed good correlation to the routinely used product formation approach. Km values were in the low micromolar range, compared to glucuronidation with Km values in millimolar ranges, indicating high affinity towards sulfation and therefore its importance as the major phase II metabolic detoxification step. Whether the differences in R/S ratios of DHEA and HMEA sulfates are potential markers for MDEA ingestion time have to be evaluated in further in vivo studies.

Keywords: MDEA, Phase II Metabolism, Stereoselectivity

S26

In Vitro Cytochrome P450 Inhibition Cocktail Assay - Part I: Development and Validation of the Liquid Chromatography-High Resolution-Mass Spectrometry (LC-HR-MS) Approach for Quantification of Nine Model Substrate Metabolites in the Incubation Mixture

Julia Dinger*, Markus R. Meyer, Hans H. Maurer; Department of Experimental and Clinical Toxicology, Saarland University, Homburg (Saar), Germany

Introduction: In vitro enzyme inhibition assays are common approaches for testing the cytochrome P450 (CYP) inhibition potential of drugs (of abuse). In contrast to marketed medicaments, drugs of abuse are not tested for inhibition before distribution. However, such inhibition may lead to drug-drug interactions that can be relevant for assessing clinical and forensic toxicology cases (e.g. Gasche et al.; N Engl J Med; 2004).

Objectives: The aim of this study was to develop and validate an LC-HR-MS method for quantification of nine CYP model substrate metabolites in the incubation mixture.

Methods: The method was developed for the following FDA-preferred and acceptable CYP test substrates and metabolites: phenacetin; acetaminophen (CYP 1A2); nicotine; cotinine (2A6); bupropion; hydroxy-bupropion (2B6); amodiaquine; deethyl-amodiaquine (2C8); diclofenac; 4-hydroxy-diclofenac (2C9); omeprazol; 5-hydroxy-omeprazol (2C19); dextromethorphan; dextrorphan (2D6); chlorzoxazone; 6-hydroxy-chlorzoxazone (2E1); testosterone; 6β-hydroxy-testosterone (3A4). For stock solutions (10 mM), all substrates and metabolites were solved in methanol or acetonitrile in accordance to their solubility and stability. The calibration and quality control (QC) sample solutions were spiked into the incubation mixture containing the corresponding microsomes, regenerating system, superoxide dismutase, and phosphate buffer (according to Meyer et al.; JMS; 2012). The samples were analyzed after protein precipitation with ice cold acetonitrile containing the internal standard (diphenhydramine) using a QExactive (ESI+/-; SIM with multiplexing; Thermo Fisher Scientific; TF; Dreieich; Germany) with gradient elution (water with 0.1% formic acid; acetonitrile with 0.1% formic acid) on a TF Accucore RP MS column (150x2.1; 2.6 µm). The analytes were analyzed in the positive mode, but hydroxy-chlorzoxazone and hydroxy-diclofenac in the negative mode. The method was validated with respect to selectivity; matrix effect; recovery; accuracy and precision.

Results: All test substrates and metabolites were sufficiently separated within 11 min. Recovery was between 74% (cotinine) and 103% (acetaminophen). Selectivity problems could not be observed. As the matrix effects ranged from 92 to 133%, calibration in matrix was mandatory. Six-point calibration (from 1% to 20 % of the literature-based Km value of the model substrate) was linear. The highest calibrator was chosen according to the fact; that expected turn-over rate in enzyme incubations should not be higher than 20% of substrate concentration. The QC samples could be quantified accurately.

Conclusion and Discussion: The developed approach allowed accurate and precise quantification of the metabolites of the model substrate of the nine cytochrome P450 enzymes mainly involved in the metabolism of drugs (of abuse). It should therefore be suitable to study the inhibition potential of drugs of abuse. Of course; this general approach must be tested for interferences with the individual drugs.

Keywords: Cytochrome P450, Inhibition, Assay LC-HR-MS

S27

Determination of Designer Drugs in Post-Mortem Blood by LC-QQQ-MS/MS Analysis

Madeleine J. Swortwood*[1], Diane M. Boland[2], and Anthony P. DeCaprio[1]; [1]Department of Chemistry and Biochemistry and International Forensic Research Institute, Florida International University, Miami, FL, USA, [2]Miami-Dade Medical Examiner Department, Toxicology Laboratory, Miami, FL, USA

Introduction: "Bath salts" and "legal highs" containing cathinone and other phenalkylamine derivatives are the newest additions to the already growing list of designer drugs. Fatalities related to the abuse of such compounds have been on the rise and validated analytical methods are necessary to screen for and confirm the presence of such drugs in both ante- and post-mortem specimens.

Objective: The purpose of this study was to assess the applicability of a LC-QQQ-MS/MS analytical method in forensic cases for the confirmation and quantification of designer drugs. The method was validated for the comprehensive analysis of multiple designer drug classes in a single analytical run with low LOQ. Following initial drug screens, the method was employed to analyze post-mortem specimens from two cases suspected of involving various designer drug entities.

Methods: An Agilent 1290 Infinity Binary Pump coupled to an Agilent 6460 triple quadrupole LC-MS/MS with Jet Streaming technology and electrospray ionization (ESI) was used for analysis. Separation occurred on an Agilent Zorbax Rapid Resolution HD Eclipse Plus C_{18} threaded column (50 x 2.1 mm; 1.8 µm particle size). Extraction of blood was performed using mixed-mode solid-phase extraction cartridges with hydrophobic C_{18} and cation exchange sites (Restek SPE Drug Prep I; 3 mL; 200 mg). Data acquisition was performed in MRM mode with positive ESI using one principal MRM transition for quantification and 1-2 additional transitions to serve as qualifiers. Method validation parameters, including selectivity, matrix effects, recovery, process efficiency, stability, linearity, precision, and accuracy, were fully evaluated according to international guidelines recommended by Frank T. Peters. The validated method was then applied to confirmation and quantification of targeted analytes in heart blood specimens from two post-mortem cases.

Results: The validation parameters of the assay satisfied all necessary acceptance criteria. The targeted compounds included 24 phenethylamines (including MDPV and 8 cathinone derivatives); and 4 piperazines. In addition, 4 tryptamines were added to the method for confirmation/quantification of a total of 32 analytes. The concentrations of drugs found in the post-mortem cases are summarized as follows - Case 1: Methylone (63 ng/mL) and MDMA (58 ng/mL); Case 2: BZP (>250 ng/mL); MDA (36 ng/mL); MDMA (115 ng/mL); MDPV (11 ng/mL); 5-MeO-DiPT "Foxy"? (>250 ng/mL); and TFMPP (93 ng/mL). The method was able to identify MDPV in Case 2, an analyte missed on initial drug screens; thus demonstrating the sensitive nature of the technique. The quantitative results for both cases were consistent with the presumptive and confirmatory results that had been previously obtained by GC-MS.
Conclusions: The developed LC-MS/MS method is suitable for the ultra-trace screening and analysis of multiple designer drug entities; including the most recent cathinone derivatives; in human blood. The validated method was successfully employed for the confirmation and quantification of several designer drugs in two post-mortem cases.

Keywords: Designer Drugs, LC-MS/MS, Validation, Post-Mortem

S28

Analysis of Benzodiazepines Including Phenazepam and Z-Drugs in Whole Blood Using an Agilent 6430 Triple Quadrupole Mass Spectrometer

Jason S. Hudson*[1], James W. Hutchings[1], Curt E. Harper[1], Patrick N. Friel[2]; [1]Virginia Department of Forensic Science, Richmond, VA, USA, [2]Agilent Technologies, Wilmington, DE, USA

Introduction: Benzodiazepines and Z-drugs are commonly encountered in forensic toxicology casework. They have indications for use in the treatment of anxiety, seizures and sleep disorders. However, they are often abused or misused and the quantitation of these drugs has become increasingly important. A single method for the detection and quantitation of these drugs would have great utility for the forensic toxicology laboratory.

Objectives: To develop and validate a method for the simultaneous detection and quantitation of 22 benzodiazepines and Z-drugs in whole blood using LC-MS/MS. The target compounds were 7-aminoclonazepam; 7-aminoflunitrazepam; chlordiazepoxide nordiazepam; flurazepam; 2-hydroxyethylflurazepam; phenazepam; diazepam; alpha-hydroxyalprazolam; alpha-hydroxymidazolam; alpha-hydroxytriazolam; midazolam; alprazolam; triazolam; oxazepam; lorazepam; clonazepam; flunitrazepam; temazepam; zopiclone; zolpidem; and zaleplon.

Methods: Whole blood samples were extracted using 1-chlorobutane under basic conditions. Extracts were reconstituted in methanol and the samples were separated using an Agilent Technologies 1260 Infinity Series LC with a Poroshell 120 EC-C18, 2.7um; 2.1x 75mm column. The samples were eluted using a gradient of 0.1% formic acid in water and 0.1% formic acid/acetonitrile with a run time of 12 minutes and then analyzed using an Agilent Technologies 6430 triple quadrupole mass spectrometer. The method development consisted of optimization of chromatography and Masshunter Optimizer® was used to identify the transitions to be used as quantitation and qualifier transitions. A thorough validation of the method was conducted that evaluated linearity; between- and within-day accuracy and precision; interferences; sensitivity; calibration models; carryover; ion suppression; robustness; stability; and previously analyzed case samples.

Results: The calibration range for all targets was 0.01-2.0 mg/L. Accuracy samples were fortified at low, medium, and high concentrations and the back-calculated concentrations were required to be within 20% of the target concentration. All targets met these criteria with the exception of midazolam that had between run accuracies of 22% for the 0.25mg/L fortified sample. Precision was assessed using triplicates of the accuracy samples with between-run precision CVs of 3-15% and within-run precision CVs of 1-18%. LOD ranged from 0.001-0.005 mg/L and the LOQ was 0.01mg/L for all targets with the exception of midazolam; zopiclone; and flurazepam. No interferences were detected after analyzing 10 drug classes (46 different drugs) and 13 matrices. Carryover was not detected up to a concentration of 7.0mg/L and dilution integrity was determined to be optimal at 1:2. Extracted samples were stable over 7 days and ion suppression and enhancement was determined to be within ±10%. Previously analyzed/simulated case samples revealed that all targets met the acceptance criteria of within 20% the target concentrations for 75% of the samples with the exception of 7-aminoclonazepam; 7-aminoflunitrazepam; chlordiazepoxide; and midazolam.

Conclusions: 7-aminoclonazepam and 7-aminoflunitrazepam met qualitative acceptance criteria only. Midazolam, chlordiazepoxide, and zopiclone have known stability issues and it is recommended that these controls and calibrators be closely monitored. This method development and validation provides a rapid and sensitive technique for the detection and/or quantitation of benzodiazepines and Z-drugs by LC-MS/MS.

Keywords: LC-MS/MS, Benzodiazepines, Z-drugs, Validation

S29

Quantification of Cannabinoids and Cannabinoid Glucuronides in Urine by Liquid Chromatography Tandem Mass Spectrometry

Karl B. Scheidweiler*, Nathalie A. Desrosiers, Marilyn A. Huestis; Chemistry and Drug Metabolism, NIDA-IRP, NIH, Baltimore, MD, USA

Introduction: Cannabis, the most commonly abused illicit drug, is often present in forensic, workplace and driving under the influence of drugs (DUID) cases. We conduct controlled drug administration studies investigating utility of urinary cannabinoid glurcuronide metabolites for documenting recency of cannabis intake.

Objective: To develop and validate a liquid chromatography tandem mass spectrometric (LCMSMS) method for quantifying delta9-tetrahydrocannabinol (THC), 11-hydroxy-THC (11-OH-THC) and 11-nor-9-carboxy-THC (THCCOOH), cannabidiol (CBD), cannabinol (CBN), THC-glucuronide and THCCOOH-glucuronide in urine.

Methods: 0.5 mL human urine was diluted with 0.5 mL pH 6.4 buffer (200 mM ammonium acetate containing 25mM dibutylammonium acetate) prior to extraction on supported liquid extraction cartridges (SLE+; Biotage; Inc.). Specimens were eluted with 3 mL ethyl acetate and dried under nitrogen. Samples were reconstituted in 150 µL mobile phase consisting of 70% A (10 mM ammonium acetate; pH 6.15) and 30% B (15% methanol in acetonitrile). 25 uL was injected onto an LCMSMS instrument consisting of a Shimadzu UFLCxr system and an ABSciex 3200 Qtrap mass spectrometer with an electrospray source. Gradient chromatographic separation was achieved utilizing a Restek Ultra Biphenyl HPLC column (100 x 2.1 mm; 5 µm particle size) with a 0.4 mL/min flow rate and an overall run time of 16 min. Quantification was conducted in MRM mode with THC-glucuronide; THCCOOH-glucuronide; 11-OH-THC; THCCOOH and CBD ionized via negative polarity while CBN and THC were ionized by positive polarity. Commercially-available deuterated internal standards were utilized; none were available for CBN, THC-glucuronide and THCCOOH-glucuronide. Therefore, THC-d_3 was utilized for CBN and THCCOOH-d_9 for THC-glucuronide and THCCOOH-glucuronide.

Results: Linear ranges were 0.5-50 ng/mL for THC-glucuronide; 1-100 ng/mL for THCCOOH; 11-OH-THC and CBD; 2-100 ng/mL for THC and CBN; and 5-500 ng/mL for THCCOOH-glucuronide ($r2 > 0.99$). Validation parameters were evaluated at three concentrations spanning linear dynamic ranges. Inter-day recovery (bias) and total imprecision (N=20) were 80.5-118.0% and 3.0-10.2% relative standard deviation; respectively. Extraction efficiencies (N=4) were 34-54% for THC, CBD and CBN, and 48-73% for the remaining analytes. Matrix effects (N=4) ranged from -10-89%. Analyte stability was assessed after 24 h on the 4˚C autosampler; 20 h at room temperature; 72 h at 4˚C; and three freeze-thaw cycles. All analytes were stable in 4˚C autosampler for 48 h. 11-OH-THC, THCOOH, THC-glucuronide and THCCOOH-glucuronide were stable under all conditions; THC, CBD and CBN showed >20% loss after 20 h at RT and after 3 freeze-thaw cycles. THC and CBN showed >20% losses after 72 h at 4˚C while all other analytes were stable.

Conclusion: We present a novel analytical method for simultaneous quantification of Phase I and II cannabinoid metabolites in a single urine extract. This method will be employed during ongoing cannabinoid administration studies and will be useful for assessing urinary cannabinoid concentrations in forensic, workplace and DUID cases. Our preliminary stability results suggest that storage conditions can confound interpretation of THC, CBD and CBN urine concentrations.

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

Keywords: Cannabinoids, Glucuronides, LCMSMS, Urine

S30
Abstract Withdrawn

S31

The Validation of Immunalysis® HEIA Urine Assays for the Screening of Various Pain Management Drugs in Oral Fluid

Gregory Sarris*; College of Pharmacy and Allied Health Professions, St. John's University, Jamaica, NY, USA

Introduction: A series of methods were developed and validated to qualitatively screen oral fluid samples for the pain management drugs buprenorphine, carisoprodol, fentanyl, and tramadol using a homogenous enzyme immunoassay technique. Commercially available kits, supplied by Immunalysis Corporation, are currently used to qualitatively test for these drugs in urine samples on the Olympus AU platform.

Objective: The intent of each validation study was to demonstrate that by manipulating certain assay parameters, these kits could be suitable for oral fluid screening, and that the existing sensitivity limits of these homogenous enzyme immunoassays could be improved.

Methods: Studies including intraday precision (n=8), inter-day precision (n=80), ruggedness, and stability were performed for each assay using certified calibrators and controls at concentrations of 0%, 50%, 100%, and 200% the established cutoff. Further studies such as EMIT/LC-MS agreement, limit of detection, interference, and selectivity were performed using blind patient samples containing unknown concentrations of each drug.

Results: All assays produced satisfactory results for the intraday and inter-day studies, indicating that each assay demonstrated the ability to qualitatively distinguish between the various calibrator and control concentrations. For the EMIT/LC-MS agreement study, a total of 153, 172, 187, and 180 patient samples were analyzed for the buprenorphine, carisoprodol, fentanyl, and tramadol assays, respectively. Values for specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV) were calculated based on the rate of true and false positive and negative results given by the immunoassay, when compared to LC-MS data. With the exception of fentanyl, all calculated values were above 85%. The limits of detection for each assay were 1.2, 12.6, 0.4, and 11.7 ng/mL, respectively. The interference and selectivity studies demonstrated that none of the assays produced false positive results from the presence of compounds commonly encountered in oral fluid such as nicotine, cotinine, caffeine, or any endogenous components. Stability studies indicated that with the exception of fentanyl, assay results were not affected when calibrator and control material was subjected to repeated freeze-thaw cycles, short term (eight hour) storage at room temperature, or long term (two week) storage under freezer conditions. The ruggedness study design is currently under development.

Discussion/Conclusions: Resulting data from each study indicated that the buprenorphine, carisoprodol, and tramadol assays are reliable methods, having suitable sensitivity and specificity for oral fluid screening. Although the fentanyl assay produced data which suggests it has utility as a valid screening method, the current lack of specificity and stability needs to be addressed. The numerous false positive results seen in the EMIT/LC-MS agreement study indicate that there may be compounds other than fentanyl, as yet undetermined, which could react with the antibodies in the assay. In addition, the results of the stability study showed that under the normal specimen storage conditions noted above, fentanyl is unstable, allowing for false negative results in the routine screening of oral fluid samples.

Keywords: Immunoassay Screen, Pain Management, Method Validations, Oral Fluid

S32

Elimination of Common Interferences by MDGCMS in the Identification and Quantification of Fentanyl and Norfentanyl in Urine

Carl E. Wolf*[1], Paul V. Macek[2], Alphonse Poklis[1]; [1]Department of Pathology, Virginia Commonwealth University, Richmond, VA, USA, [2]Shimadzu Scientific Instruments, Inc., Columbia, MD, USA

Introduction: We present a Multi-Dimensional Gas Chromatography Mass Spectrometry (MDGCMS) method for the identification and quantification of fentanyl and its metabolite, norfentanyl, in urine. The MDGCMS was configured so both the analytical and the “cut" column were plumbed into the same MS. Thus, the MDGCMS can be used as a GCMS and/or a MDGCMS in the same analytical run. The MDGC separates fentanyl and norfentanyl from common co-eluting interferences (e.g.; phthalates) present in human urine specimens due to the normal collection of urine in plastic urine containers. The ability of the MDGC to make “heart cuts" from the analytical column and divert the cut to a second column to separate norfentanyl and phthalates, improves the identification and sensitivity of the method, and reducing the sample volume requirement and sample preparation costs.

Methods: The MDGCMS was a Shimadzu system consisting of a 2010 Ultra MS connected to two Shimadzu GCs and a “Deans" switch. The GC1 column was an Rtx-5 (20 m x 0.18 mm ID x 0.2 um df); the GC2 column was an Rtx-50 (10 m x 0.18 mm ID x 0.2 um df); and the mid-pipe was deactivated fused silica (1 m x 0.15 mm ID) (Restek Corporation; Bellefonte; PA). Fentanyl, norfentanyl and their respective deuterated internal standards were extracted from 1 ml of urine using solid phase extraction (MP3 column; Agilent Technologies; Wilmington; DE). A propionic anhydride derivative of norfentanyl was prepared.

Results: The method validation was assessed over five days. The analytical measurement ranges for fentanyl and norfentanyl were 0.5 -œ 1000 ng/ml and 0.5 -œ 5000 ng/ml; respectively. The limits of detection and quantitation were set at 0.5 ng/ ml for fentanyl and norfentanyl. Concentrations of 5 and 100 ng/ml were used to evaluate matrix effect; recovery and process efficiency; which were: fentanyl (5 ng/ml) 121%; 79%; 96%; (100 ng/ml) 109%; 73%; 79%; norfentanyl (5 ng/ml) 135%; 77%; 104%; (100 ng/ml) 118%; 82%; 97% respectively. Interference from drugs and biological constituents was assessed; no interferences were noted. Analysis of 30 previously analyzed urine specimens; with concentrations ranging from 0.5 -œ 400 ng/ml fentanyl and 2 -œ 4800 ng/ml norfentanyl; yielded correlations of 98% and 96%; respectively.

Conclusions: We present a simple, reliable and interference-free method for the determination of fentanyl and norfentanyl in urine.

Keywords: Fentanyl, Norfentanyl, MDGCMS, Urine

S33

Development of a New Gas Chromatographic Column Set for the Analysis of Blood Alcohol Concentration

Frank Dorman[1], Jessica Westland[1], **Amanda Rigdon*[2],** Jarl Snider[2], Richard Morehead[2], Chris Denicola[2]; [1]Pennsylvania State University, State College, PA, USA, [2]Restek, Bellefonte, PA, USA

Introduction: Blood alcohol concentration (BAC) analysis is one of the most common analytical tests performed by laboratories involved in forensic and medical testing. Chromatographic separation of the target analytes, and possible interfering compounds is critical for the analysis. Incomplete chromatographic separation will cause quantification bias, and possibly invalidate the results of the test. This condition makes a number of demands on laboratories performing this work.

Objective: The objective of this work was to develop a set of analytical columns suitable for the analysis of BAC samples through the use of stationary phase modeling techniques such as window diagramming and computer-assisted stationary phase design (CASPD) modeling. The main requirement of the new column set was to perform better than existing BAC columns, while accommodating the use of either 1-propanol or the newer tert-butanol internal standard, which experiences coelutions on some BAC columns.

Methods: A standard containing methanol, ethanol, 1-propanol, acetaldehyde, acetone, isopropanol, and tert-butanol was analyzed using two different isothermal oven temperatures (35°C and 50°C) on methyl, phenyl, diphenyl, and fluoro-based stationary phases to generate thermodynamic indices and retention time data for window diagramming and CASPD modeling. From this data, experimental stationary phase compositions were determined that were predicted to resolve compounds of interest. These stationary phases were synthesized and prototype columns were made. The prototype columns, as well as several currently available BAC columns, were tested using a 0.4g/dL mixture of acetaldehyde, methanol, ethanol, isopropanol, acetone, 1-propanol, tert-butanol, acetonitrile, ethyl acetate, and methyl ethyl ketone. Analysis was performed using Tekmar HT3 headspace autosampler and an Agilent 6890 GC-FID. Samples were incubated at 60°C for 5 minutes, and analyzed isothermally on 0.53mm ID columns for each prototype stationary phase at 40°C. Injector and detector temperatures were 200°C and 240°C, respectively.

Results: Baseline resolution was achieved for all compounds on both column phases. Enhanced resolution between ethanol and all other compounds of interest was observed. The new columns were also tested for bleed, lot-to-lot reproducibility, and robustness. Bleed for both analytical columns was < 15pA at 240°C. Lot-to-lot reproducibility testing (n = 3 lots, 87 columns) for k' values and retention indices showed < 1.2% RSD for all parameters tested. Robustness testing involved +/- 5°C changes in oven temperature, and +/- 10% linear velocity of the carrier gas versus the 80cm/sec standard method. Baseline resolution was maintained for all compounds under all conditions tested.

Conclusion/Discussion: Modeling the performance of applicable test probes on several different stationary phases allowed for development of a novel BAC column set that supports the use of either 1-propanol or tert-butanol internal standards. Additionally, enhanced resolution between ethanol and other compounds of interest allows for greater confidence in analytical results, and greater ease in reporting. Additionally, through the optimization of other method parameters such as linear velocity, liner choice, split ratio, and injection volume, improved method performance was achieved. These method parameters and the factors affecting them will also be discussed, to help the audience improve their blood alcohol analyses regardless of instrument platform or choice of column.

Keywords: Blood Alcohol, DUI, GC, Headspace

S34

Development of a Biochip Array for the Simultaneous Screening of Fifty Eight Designer Drugs in Whole Blood and Urine

J. Darragh*, J. Porter, W. Snelling, V. Anderson, V. Elder, E. McShane, R. McCreanor, R.I. McConnell, S.P. Fitzgerald; Randox Toxicology Limited, Crumlin, UK

Background: 'Designer drugs' is the term used to describe drugs manufactured to avoid current drug laws and methods of detection. Frequently marketed as 'herbal highs', abuse of these compounds is high due to their perceived 'natural' origin and safety profile. The introduction of new designer drugs is evolving continuously and as such the availability of immunoassay screens for these compounds is limited. Evidence biochip array technology provides a platform for the simultaneous determination of multiple analytes from a single sample. This leads to an increase in the results output and a reduction in the consumption of the sample/reagent. Due to the extensive number of designer drugs currently on the market, immunoassays presenting wide specificity are of interest in test settings.

Objective: This study reports, to our knowledge, the first biochip platform for the multiplex screening of a wide range of 'Designer Drugs': ‘bath salts’, synthetic cannabinoids ('Spice'), phenylpiperazines, benzylpiperazines ('PEP pills'), salvinorin and mescaline in whole blood and urine.

Methods: The core of this technology is the biochip (9mm x 9mm), which represents the chemically activated solid phase where the ligands are immobilized and stabilized; defining microarrays of test sites and also the vessel for the immunoreactions. For the screening of designer drugs on this biochip platform, 11 test sites corresponding to competitive chemiluminescent simultaneous immunoassays are applied using dedicated reagents for both whole blood and urine. The Evidence Investigator analyser was used. The system incorporates dedicated software to process, report and archive the data generated.

Results: The four immunoassays for synthetic cannabinoids on the biochip detected thirty-two compounds including JWH-018, JWH-073, JWH-081, JWH-250 and RCS-4 with % cross-reactivities ranging from 80 (JWH-081) to 300% (JWH-073). Twelve phenylpiperazine and benzylpiperazine compounds were detected across three immunoassays. Two 'bath salts' immunoassays detected ten different compounds including mephedrone, methcathinone, MDPV and MDPBP. The Salvinorin assay (standardized to salvinorin A) also detected the deacetylated form salvinorin B. The limits of detection (LOD) were as follows: the four immunoassays for synthetic cannabinoids ranged from 1.4 to 7.6ng/ml (urine) and from 0.5 - 3.6ng/ml (whole blood). For the two’ bath salts’ immunoassays, LOD ranged from 0.4ng/ml-2.5ng/ml (urine) and 0.03-1.2ng/ml (whole blood). Phenylpiperazines LODs ranged from 2.3-4.9ng/ml (urine) and 0.07-0.85ng/ml (whole blood), and benzylpiperazines 1.3ng/ml (urine) and 1.4ng/ml (whole blood). The LOD's of salvinorin and mescaline were 0.3ng/ml (urine) 0.09ng/ml (whole blood) and 6.7ng/ml (urine) and 0.3ng/ml (whole blood) respectively.

Conclusion: The data indicates that the developed biochip array incorporates simultaneous immunoassays with different specificity profiles which allow the multiplex screening of fifty-eight 'Designer Drugs' from a single sample of urine or whole blood. Biochip array technology represents a flexible multi-analytical approach as test sites for new drugs can be incorporated to the arrays. This has important applications in forensic toxicology and workplace drug testing. This methodology is a very applicable tool for the rapid screening of batches of samples, as only positive sample determinations require further confirmatory analysis.

Keywords: Designer Drugs, Biochip Array, Synthetic Cathinones, Synthetic Cannabinoids, Mescaline

S35
Synthetic Cannabinoids in Blood and Urine: An Update

Sherri L. Kacinko, **Barry K. Logan***, NMS Labs, Willow Grove, PA, USA

Introduction: Synthetic cannabinoids continue to present a challenge to the toxicology community. Manufacturers and distributors are constantly updating the contents of their herbal blends in order to stay ahead of local and federal legislation. Data from blood specimens analyzed from Sep 27, 2010, to Jan 31, 2012, and urine specimens analyzed from Sep 15, 2011, to Jan 31, 2012, were evaluated to monitor positivity rates and identify trends.

Methods: Data was extracted from the laboratory information management system. Initially blood analysis included JWH-018, JWH-019 (qualitative only), JWH-073 and JWH-250. In July 2011 the method was updated to include 8 additional analytes, AM-2201, JWH-200 and RCS-4 quantitatively and AM-694, JWH-081, JWH-122, JWH-210, and RCS-8 qualitatively. Initial urine testing included only JWH-018 and JWH-073 metabolites; however, metabolites of three additional compounds were quickly added as blood analysis indicated their popularity. The current urine test included the qualitative identification of 4-OH-JWH-018, 5-OH-JWH-018, 3-OH-JWH-073, 4-OH-JWH-073, 4-OH-AM-2201, 4-OH-JWH-250 and 5-OH-JWH-019. These data were grouped by parent compound (JWH-018, JWH-073, JWH-250, JWH-019, and AM-2201) for analysis.

Results/Discussion: A total of 896 blood cases were available for examination; 228 of which were analyzed on the four analyte panel. Since the beginning of testing, the average montly positivity rate was 47 ± 14% Prior to becoming federally controlled, JWH-018 was the primary analyte detected with a maximum positive rate of 61% in March 2011. This rate dropped to 31% in April and has averaged 12% (9.5 - 14%) since June 2011. From July-Sep 2011, JWH-122 was the most prevalent analyte and starting in Oct2011 AM-2201 is has the highest positivity rate. No samples have tested positive for AM-694 or JWH-200. JWH-210 has positivity rates around 15%, and all other analytes have currently rates <10%.

Since Sep 2011, 4732 urine specimens have been analyzed with 32% testing positive for at least one analyte. Positivity rates decreased 55% between Sep 2011 and Jan 2012; however, the total number of specimens analyzed increased by over 400% in the same time period. Like blood, JWH-018 is the most commonly represented analyte. Positivity rates for 4-OH-JWH-018 and 5-OH-JWH-018 have decreased from 15 to 3.4% and 29 to 16%, respectively. Based on the structures of AM-2201 and JWH-018, it is hypothesized that 5-OH-JWH-018 is also a metabolite of AM-2201. This is supported by positivity rates of 4-OH-AM-2201 that are similar to 5-OH-JWH-018 and the fact that 5-OH-JWH-018 is always positive if 4-OH-AM-2201 is positive.

Conclusions: Based on the current analysis rate of approximately 100 cases/month it is estimated that an additional blood 400 cases will be included in the final analysis. The number of urine cases analyzed each month continues to increase; 2805 urine specimens were received for analysis in Feb 2012. Based on these numbers, it is estimated that at least 8000 more cases will be included in the final data. In addition, the metabolites of four additional synthetic cannabinoids, including JWH-122 and JWH-210, are being added to the urine. It is expected that with the addition of these metabolites, the positivity rates in urine will increase.

Keywords: Synthetic Cannabinoids, Blood, Urine

S36

Intoxication of a Young Girl with JWH-210 and AM-2201 After Smoking an Herbal Blend

Andreas H. Ewald*[1], Nadine Schaefer[1], Benjamin Peters[1], Markus R Meyer[2], Hans H. Maurer[2], Peter Schmidt[1]; [1]Institute of Legal Medicine, Saarland University, Homburg (Saar), Germany, [2]Department of Experimental and Clinical Toxicology, Saarland University, Homburg (Saar), Germany

Introduction: Some years ago, psychoactive substances emerged on the market as so-called legal highs and are still present in different compositions. The ingredients of products sold as bath salts or herbal essences are often not known by the consumer as well as by the seller. But that is not the only risk of these products. Bad quality due to not controlled manufacturing processes can lead to different contents and inhomogeneous distribution of the active substances in the products.

Objectives: A girl smoked a cigarette with an herbal essence, which was advertised to calm down the consumer, together with a friend. Afterwards she was brought to the hospital complaining hallucinations, increased pulse rate and fear of a heart attack. A cellophane foil with residues of the herbal essence was seized for analysis and a blood sample was taken. The friend had no symptoms although they smoked the one cigarette together, so no blood sample was taken from her. After surveillance of the patient, she left the hospital the day after without clinical symptoms. The aim of the study was to identify and quantify possible applied cannabinoids.

Materials and Methods: The cellophane foil was washed with ethanol and this washing solution was directly analyzed by GC-MS (GC 5890 Serie II; HP MSD 5972; HP1 column) in scan mode. The blood plasma was extracted liquid-liquidly with ether/ethylacetate at pH 7.4 and 10, screened by GC-MS in scan mode and analyzed by LC-MS/MS in MRM mode for synthetic cannabinoids using an AB Sciex QTrap 3200 (ESI+) coupled with a Shimadzu Prominence HPLC. A Waters Sunfire column (C18; 150x2 mm; 3.5 µm) was used for separation with gradient elution (A: 20mM aqueous ammonium formate; pH 3.5 / B: acetonitrile; 0.1% formic acid).

Results: In the qualitative GC-MS scan of the washing solution JWH-210 and AM-2201 could be detected. In the blood plasma sample JWH-210 and AM-2201 could also be found. The amounts in the plasma were approximately 5 ng/ml of JWH-210 and approximately 2 ng/ml of AM-2201. No other relevant substances could be found in the blood plasma sample.

Conclusion and Discussion: In the present case, the synthetic cannabinoids JWH-210 and AM-2201 could be identified in the washing solution of the cellophane foil and in the blood sample. The semi quantitative results of JWH-210 and AM-2201 in the plasma can be assessed as very high compared to THC considering the about 100 and about 50 fold stronger binding to cannabinoid receptors, respectively. So the intoxication symptoms may result due to synthetic cannabinoid intake. Lacking symptoms of the friend, who smoked the same cigarette, may be explained by possible inhomogeneous distribution of the active substances as seen in other seized material; or maybe habituation of the friend to cannabinoids.

Keywords: JWH-210, AM-2201, Legal High, Intoxication

S37

Simultaneous Analysis of 7 Synthetic Cannabinoids: JWH018, JWH073, JWH250, JWH210, JWH081, RCS-4, AM2201 and Their Contribution to the Overall Positivity in Routine Oral Fluid Specimens

Sumandeep Rana*, Tara Brunson, Wayne B. Ross; Redwood Toxicology Laboratory, Santa Rosa, CA, USA

Introduction: In March 2011, the United States Drug Enforcement Agency banned five synthetic cannabinoids including JWH018 and JWH073, making them Schedule 1 controlled substances. Redwood Toxicology laboratory has been testing for these and JWH250 in oral fluid since Nov 2010 and noted a sudden decrease in positivity following the ban. Analysis of various synthetic cannabinoid laced herbal preparations post ban revealed the changing active ingredients. Based on the results, analytical procedure for oral fluid was updated to include currently un-scheduled drugs: JWH210; JWH081; RCS4 and AM2201. Over 400 authentic oral fluid specimens were re-analyzed using the expanded method.

Objective: To evaluate the concentrations and contribution of currenty un-scheduled drugs towards the overall positivity rate in oral fluid. Concentrations of JWH018, JWH073 and JWH250 found routinely in oral fluid have been previously reported. This study reports the concentrations of other synthetic cannabinoids covered in the procedure.

Methods: Oral fluid specimens were collected using Quantisal collection device resulting in a 1:4 dilution. Extensive extraction efficiency, matrix effect and specificity studies were carried out during method validation. LOD and LOQ for all analytes were found to be 0.1 and 0.25ng/mL respectively. 100 µl of sample was removed from the collection tube, diluted with dimethylformamide and centrifuged. 20µL of the prepared sample was injected on to a Pinnacle DB biphenyl, 5µ x 50mm x 2.1mm column with a flow rate of 0.5 mL/minute. Mobile phases were 0.1% formic acid with 2mM ammonium formate and 0.1% formic acid with 2mM ammonium formate in acetonitrile. The gradient started at 10% organic and increased to 90% organic in 8 minutes before returning to original conditions. Method was applied to 411 authentic oral fluid specimens with an administrative cut-off of 0.25ng/mL.

Results: Extraction efficiency of all seven analytes was >66%. Of the 411 specimens analyzed, 60 (15%) were positive. JWH018 was no longer the most frequently found analyte as previously reported. The new most frequently found drug, AM2201, was detected in all (100%) of the positive specimens either alone (33%) or in combination with other drugs (67%). JWH210, JWH018, JWH250, JWH073 and JWH081 were detected in 47%, 45%, 10%, 7% and 4% of the positives, respectively. RCS4 was not found in any specimen. Concentrations in positives ranged from 0.25-332ng/mL for AM2201, 0.25-54ng/mL for JWH210, 0.25-51ng/mL for JWH018, 0.25-1.68ng/mL for JWH250 and 0.25-223ng/mL for JWH081.

Conclusions: Routine analysis of synthetic cannabinoids in biological matrices is a moving target. Clandestine “Drug Chemists" are quick at changing the active ingredients to circumvent regulations. Prior to the DEA scheduling of five synthetic cannabinoids including JWH018 which was the most commonly used, the average positivity rate in oral fluids was 15% with a three drug panel monitoring JWH018, JWH073 and JWH250. Soon after the ban, average positivity rate dropped to 4%. Expanding the testing to include AM2201 and JWH210 restored the positivity back to 15%. Oral fluid appears to be an ideal test matrix for the newly emerging synthetic cannabinoids as new regulations are being imposed and the product compositions changing to circumvent the control.

Keywords: Synthetic Cannabinoids, Oral Fluid, LCMSMS

S38

A Fatal Poisoning Involving the Synthetic Cannabinoids JWH-122 and JWH-210

Daniela Remane*[1], Volker Aurter[2], Rolf Werner[1], Julia Schermer[1], Stefanie Drobnik[1], Gita Mall[1], Frank T. Peters[1]; [1]Institute of Forensic Medicine, Jena University Hospital, Jena, Germany, [2]Institute of Forensic Medicine, University Medical Center Freiburg, Freiburg, Germany

Introduction: Synthetic cannabinoids are an emerging drug class easily available over the internet as herbal incense or smoking blends. Although the toxic effects of the synthetic cannabinoids are not yet fully evaluated, they were associated with symptoms such as convulsions and heart failure.

Objective: To report a fatal case involving the synthetic cannabinoids JWH-122 and JWH-210.

Case History: A 25-year-old male was found dead at home around noon. He laid on the ground in a cramped position with foam on both nose and mouth. Apart from some over-the-counter cold medications, smoking accessories and a package of the herbal smoking blend “Wasted" were found in the apartment. According to his girl friend, the deceased had smoked a cigarette containing parts of the “Wasted"? mixture the evening before. It is unknown whether or not he smoked more of the drug shortly before death. The deceased had a history of alcohol, nicotine, and drug abuse and reportedly suffered from a borderline personality disorder.

Methods / Toxicological Analysis: The supernatant of a methanolic suspension of the green tablet was analyzed by fullscan gas chromatography-œmass spectrometry (GC-œMS) after acetylation; high-performance (HP) liquid chromatography (LC) with diode array detection (DAD); and LC-tandem mass spectrometry (MS/MS). Immunoassay-based drug screening for the common drugs of abuse was performed in a postmortem urine specimen. After enzymatic conjugate cleavage, a urine sample was further worked up by liquid-œliquid extraction (LLE) and acetylation and analyzed by fullscan GC-œMS. Heart blood was submitted to LLE followed by HPLC-DAD analysis. Gastric content and femoral blood were analyzed with fullscan GC-MS after LLE. Target analysis for 25 synthetic cannabinoids was performed in femoral blood using LC-MS/MS after LLE. Finally, alcohol concentrations were determined in femoral blood and urine using GC with flame ionization detection.

Results:

Autopsy Finding- External examination revealed no relevant signs of harm or physical violence. In the hair of the deceased, parts of a green tablet were found. Internal examination of the body revealed unspecific findings with obvious pulmonary and cerebral edema, and congestion of inner organs with blood.

Histological Finding- Histologic examination with hematoxylin and eosin staining confirmed congestion and edema of lung and brain as well as congestion of heart, liver, spleen, and kidneys.

Toxicological Finding- With the exception of nicotine and caffeine, no common drugs, pharmaceutical agents or alcohol could be detected in the analyzed urine and blood samples. The GC-MS and the targeted LC-MS/MS analysis for 25 synthetic cannabinoids were negative for the tablet suspension. In the analyzed femoral blood sample, JWH-122 and JWH-210 were detected by LC-MS/MS. Their concentrations were estimated at 17 ng/mL and 15 ng/mL, respectively, which seems excessively high considering the high potency of the compounds.

Conclusion: After exclusion of other potential causes of death, a fatal poisoning with JWH-122 and JWH-210 could be assumed. The presented case shows that synthetic cannabinoids may lead to life-threatening poisonings which confirms the considerable toxicity of these drugs previously indicated by severe clinical poisoning cases.

Keywords: Synthetic Cannabinoid, JWH, Poisoning, LC-MS

S39

A Toxicological Approach to Confirm the Inhalation of Pyrolysis Products in Burned Cadavers Using Headspace Solid-Phase Microextraction and Cryogenic Gas Chromatography-Mass Spectrometry

Brian Waters*, Kenji Hara, Masayuki Kashiwagi, Aya Matsusue, Shin-ichi Kubo; Fukuoka University, Faculty of Medicine, Department of Forensic Medicine, Fukuoka, Japan

Introduction/Objective: Detection of pyrolysis products in the blood of cadavers that have died in a fire can have a major impact on the death investigation. It can signify whether or not the deceased was alive or dead during the fire, and if the fire was set with accelerants. Analysis of these compounds is difficult because of their volatile nature. Utilizing a headspace solid-phase microextraction (SPME) technique coupled with cryogenic gas chromatography-mass spectrometry (GC-MS), we developed an analysis to determine the presence of these volatile compounds in burned cadavers. Several actual case examples will be discussed.

Methods: In a glass vial, 0.1 g of blood was added to 1 mL of distilled water and 1 microliter of internal standard (IS). The vial was heated to 50°C for 10 min. After 2 min at room temperature, the fiber was inserted into the vial and exposed. The vial was then placed in a cold block at 0°C. After 15 min, the fiber was inserted into the injector port of the GC-MS for analysis. The instrument used was a Shimadzu QP-2010 Ultra GC-MS. A tandem capillary column composed of a Restek Rtx-5 (10 m x 0.18 mm i.d., 0.20 um) coupled with a Restek Rtx-5ms (1 m x 0.32 mm i.d., 0.5 um) was utilized. The GC-MS parameters were as follows: a splitless injection with an injector temperature of 250°C; an oven temperature program of -40°C to 290°C at 30°C/min; a helium flow rate of 2.1 mL/min; and an interface temperature of 260°C.

Results: The established method was effective in identifying a total of 30 compounds; 7 of which were deuterated internal standards. Five-point calibration curves were generated and quantitation was performed on many of the detected compounds. From this analysis we clearly detected compounds such as aliphatic and aromatic hydrocarbons that are common in kerosene and gasoline inhalation, as well as other petroleum breakdown products like styrene, indene, and naphthalene. It is common to detect smoke inhalation compounds in cases where the carboxyhemoglobin (COHb) saturation is high. However, this method detected petroleum breakdown products in cases even where the COHb saturation was low. In fact, in most cases where the autopsy findings indicated death by fire, styrene, indene, and naphthalene were detected even if the COHb saturation was low.

Conclusion: A method was developed that identified the presence of volatile hydrocarbons in a cadaver's blood to show if the victim was alive or dead during the fire. The same method identified the presence of compounds in the blood that could signal the use of accelerants.

Keywords: Pyrolysis, Fire-Related Deaths, Cryogenic GC-MS, Tandem Columns, SPME

S40

In Which Organs Can JWH-210 and JWH-122 be Detected Four Weeks After Single Drug Administration to Rats?

Nadine Schäfer*, Benjamin Peters, Dietmar Bregel, Peter Schmidt, Andreas H. Ewald; Institute of Legal Medicine, Saarland University, Homburg, Germany

Introduction: A few years ago synthetic cannabinoids such as alkylindole derivatives have established themselves on the drug market worldwide. They are often sold as herbal blends or incenses and are usually declared as “not for human consumption". In fact, they cause cannabis-like effects after smoking as they contain CB1-receptor agonists such as JWH-018. Some of these cannabimimetics were added to the controlled substances act; which led to a rising appearance of new, not scheduled, synthetic cannabinoids. Most of them can be detected in blood after recent consumption, but there are no data whether a past consumption can be detected post-mortem?

Objectives: The aim of the present work was to study in which organs JWH-210 ((4-ethylnaphthalen-1-yl)(1-pentyl-1H-indol-3-yl)methanone)) and JWH-122 ((4-methylnaphthalen-1-yl)(1-pentyl-1H-indol-3-yl)methanone)) can be detected four weeks after administration.

Materials and Methods: Extracts of herbal mixtures containing JWH-210 or JWH-122 (20 mg drug per kg body weight) were administered to rats for toxicological diagnostic reasons. After four weeks, the rats were dissected and adipose tissue, brain, liver, hindgut contents, and hairs were collected. Adipose tissue was homogenized in and extracted by acetone. The other tissues were homogenized in water and isolated by liquid-liquid extraction according to Maurer et al.; JCB; 2002. The hair samples were first washed twice with water and once with acetone, pulverized, and extracted with ether/ethyl acetate after alkaline hydrolysis with aqueous sodium hydroxide. JWH-018 was added before extraction as internal standard. The samples were analyzed by an AB Sciex QTrap 3200 LC-MS/MS (ESI+) coupled with a Shimadzu Prominence HPLC. Separation was achieved using a Waters Sunfire column (C18; 150 x 2.1 mm; 3.5 Ã‚µm) and gradient elution (acetonitrile; 0.1% formic acid/aqueous ammonium formate; pH 3.5). Detection was performed in multi-reaction monitoring (MRM) mode of three transitions of each precursor ion and an enhanced product ion (EPI) spectrum when the intensity was higher than 5000 counts.

Results: Four weeks after application, JWH-210 was detected in adipose tissue (appr. 60 ng/g); hair (appr. 4 ng/g) and in traces in the hindgut contents. JWH-122 could only be detected in adipose tissue (appr. 8 ng/g) and in traces in hair.

Conclusion: This study showed that JWH-122 and JWH-210 were detected in adipose tissue and in hair.

Keywords: JWH-122, JWH-210, Rat Tissues, LC-MS/MS

S41
Rapid Analysis of “Bath Salts" in Urine by LC-MS/MS

Matthew W. Bjergum*, Loralie J. Langman; Mayo Clinic, Rochester, MN, USA

Introduction: In recent years, a new group of designer drugs, the synthetic cathinones (beta-keto phenylalkylamines, also known as Baths Salts, have emerged. These compounds have caused frequent serious intoxications and some deaths in several countries. The primary effects tend to reflect those of sympathomimetic stimulants, like amphetamines.

Objective: We report here a simple, rapid, and robust LC-MS/MS method to identify and quantitate mephedrone, methylone, ethylone, and 3;4-methylenedioxypyrovalerone (MDPV) in urine.

Methods: 50 uL of calibration standards, quality controls, and unknown samples were mixed with 30 uL of 1.0 ug/mL dueterated internal standards and 500 uL of water containing 1% formic acid and 5% methanol. After mixing, 15 uL was injected onto an Agilent 1200 series HPLC with an Agilent 6460 LC-MS/MS using electrospray ionization in positive ion mode. The analytical column was an Agilent C18 StableBond 50 x 3.0mm; 1.8-um. The column was held constant at 50C with a flow rate of 800 uL/min throughout the analytical run. Mobile phase gradient conditions were the following: 5% MP-B from 0 - 1 min; ramp up to 95% MP-B between 1.0 - 3.5 min; 95% MP-B from 3.5 - 4.5; post time of 2.0 min to re-equilibrate at 5 % MP-B. Drugs eluted off the analytical column between 2 and 4 minutes. A quantitating and two qualifier transitions were monitored for all analytes including each internal standard. Concentrations were calculated based on a five point calibration curve using a $1/x^2$ linear curve fit.

Results: The analytical linear range was 10- 1000 ng/mL with a limit of detection of 5 ng/mL. The average (n=10) linear regression for each drug demonstrated the following: mephedrone slope= 1.02; r^2= 0.9997; and intercept= -3.59; methylone slope= 1.02; r^2= 0.9997; and intercept= -3.30; ethylone slope= 1.02; R2= 0.9998; and intercept= -3.67; MDPV slope= 1.02; r^2= 0.9998; and intercept= -3.61. With-in (n=20) and between-run (n=10) precision CVs were less than 5% across the analytical range for each drug. Analyte stability was demonstrated for up to 1 day ambient; 7 days refrigerate; 14 days frozen; and the drugs were stable for 3 freeze/thaw cycles. Absence of interferences from the top 25 prescribed drugs or common drugs of abuse including those in the amphetamine type stimulant family. Minimal ion suppression or enhancement due to the matrix effect was observed. No significant carryover was seen following a sample containing 5000 ng/mL of each drug.

Conclusion: The method described here provides law enforcement, toxicologists, and healthcare professionals a precise, rapid, and simple method to detect mephedrone, methylone, ethylone and MDPV in urine.

Keywords: Bath Salts, LC-MS/MS

S42
Analysis of Psychedelic Phenethylamines in Blood by LC/MS/MS

Sarah Martin*, Sarah Kerrigan; Sam Houston State University, Sam Houston State University, Huntsville, TX, USA

Introduction: Psychedelic phenethylamines are a class of designer drugs capable of producing a complex array of sought after adrenergic and hallucinogenic effects. Several of these synthetic psychotropics are not scheduled and bypass controlled substance legislation in the United States. Hallucinogenic phenethylamines were first synthesized by Shulgin and later emerged as illicit drugs in Europe and Asia before making an appearance in this country over the past decade. Although these drugs are seized with some frequency in the United States, they pose a number of challenges to toxicology laboratories, and are rarely reported.

Methods: In this study, fifteen designer amphetamines were simultaneously identified in blood using solid phase extraction (SPE) and liquid-chromatography tandem mass spectrometry (LC/MS/MS). Extraction, separation and detection were achieved using mixed mode polymeric SPE, C18 LC column, and positive electrospray ionization. The procedure utilized mescaline-d9 as the internal standard and consumed 1mL of whole blood. Target drugs included 2,5-dimethoxy-4-bromophenethylamine (2C-B), 2,5-dimethoxy-4-chlorophenethylamine (2C-C), 2,5-dimethoxy-4-methylphenethylamine (2C-D), 2,5-dimethoxy-4-ethylphenethylamine (2C-E), 2,5-dimethoxyphenethylamine (2C-H), 2,5-dimethoxy-4-iodophenethylamine (2C-I), 2,5-dimethoxy-4-ethylthiophenethylamine (2C-T-2), 2,5-dimethoxy-4-isopropylthiopehnethylamine (2C-T-4), 2,5-dimethoxy-4-propylthiophenethylamine (2C-T-7), 2,5-dimethoxy-4-bromoamphetamine (DOB), 2;5-dimethoxy-4-chloroamphetamine (DOC), 2,5-dimethoxy-4-ethylamphetamine (DOET), 2,5-dimethoxy-4-iodoamphetamine (DOI), 2,5-dimethoxy-4-methylamphetamine (DOM) and 4-methylthioamphetamine (4-MTA).
.
Results: Solid phase extraction (SPE) and liquid-chromatography tandem mass spectrometry (LC/MS/MS) were used for isolation and identification of drugs from whole blood. Analytical recoveries ranged from 60%-91% and limits of detection were in the range 0.5-1.0 ng/mL. Precision and accuracy was assessed at 50 and 250 ng/mL in blood. Covariance at 50 and 250 ng/mL were 1-6% and 2-6%; respectively (n=4). Accuracy for all analytes was in the range 99-112% at 50 ng/mL and 89-99% at 250 ng/mL. Matrix effects were evaluated statistically using 20 drug-free whole blood samples collected in grey-top and lavender-top tubes. No ion suppression or enhancement was present. There were no interferences from other commonly abused amphetamines and the limit of quantitation for all fifteen drugs ranged from 0.5-2.0 ng/mL.

Conclusions: Many of the psychedelic amphetamines described in this study are difficult to detect in toxicological samples due to their low dose and extensive biotransformation. A highly sensitive and specific LC/MS/MS procedure was developed to identify fifteen of the most commonly used 2C-; 2C-T and DO-series of designer drugs in whole blood.

Keywords: LC/MS/MS, Designer Drugs, Blood

S43
Abstract Withdrawn

S44
Analysis of Urine for the Detection of Kratom Use

Tania A. Sasaki*; Avee Laboratories, Clearwater, FL, USA

Background: In the continuous search for “legal highs" and/or undetectable drugs, Kratom use has increased in popularity over the past 1-2 years. Kratom is a plant, Mitragyna speciosa, native to South Asia and has historical use as an herbal drug. Although several alkaloids are present in the Kratom leaf, the major active alkaloid is believed to be mitragynine and, to a much lesser extent, 7-OH-mitragynine. It has been reported that in low doses, Kratom has stimulant-like effects. In larger doses, the effects have been reported to be more opiate-like, i.e. sedative and/or analgesic. Kratom is widely available on the internet, in convenience stores and “head-shops."? Because use of Kratom is beginning to increase, it is important to develop techniques to detect Kratom use, especially in drug abstinence programs.

Objective: The objective of this study was to detect and identify the compounds excreted in urine after Kratom use and develop a method for routine testing.

Methods: Powder from a commercial Kratom capsule was extracted with methanol and this extract utilized for initial method development. A reference standard of mitragynine was purchased from Cerilliant when it became available. Analysis was performed on an AB Sciex 3200 QTRAP mass spectrometer interfaced with a Shimadzu Prominence LC.

Results: Mitragynine was clearly detected in the pill extract and confirmed using the reference standard. The LC-MS/MS method also separated and detected the other three reported stereoisomers of Kratom: speciogynine; speciociliatine; and mitraciliatine. 7-OH-mitragynine was also detected, but at much lower concentrations. A urine sample from an admitted Kratom user was sent to the laboratory. All four stereoisomers were easily detected in the sample, as well as several hydroxyl metabolites. A preliminary study of 500 urine specimens from a random sampling of patients in substance abuse rehabilitation programs detected Kratom use in almost 4% of the specimens.

Conclusion/Discussion: Designer drugs and legal highs continue to be a challenge for toxicologists. Kratom is one of the latest drugs/plants of concern, especially as its popularity continues to grow. An LC-MS/MS method has been developed to detect use. The prevalence of Kratom use in a population required to abstain from all drugs and alcohol demonstrates the need for further research and implementation of routine testing.

Keywords: LC/MS/MS, Emerging Drugs, Kratom, Mitragynine, Legal Highs

S45

Analysis of Methamphetamine and Its Metabolite, Amphetamine by Chiral CESI-MS

John C. Hudson*, Department of Chemistry & Biochemistry, University of Regina, Regina, Saskatchewan, Canada

Introduction: Chiral separation of drug enantiomers is essential in order to show that the active enantiomer is present in forensic specimens. For example, chiral analysis of methamphetamine "street" samples yields information on the clandestine lab synthetic route. Chiral analysis is also of great importance in Pharma and Drug Discovery for the detection and quantitation of chiral impurities. In 2005, Rudaz and Veuthey (1) used a Partial Filling Technique (PFT) under countercurrent conditions, employing highly sulfated cyclodextrin additives to a simple background electrolyte (BGE) to separate the enantiomers of amphetamine derivatives. In this work, a low flow Capillary Electrophoresis Electrospray Interface for Mass Spectrometry (CESI-MS) was used with the Partial Filling Technique to generate the chiral separation for quantitative data.

Objective: To separate, identify and quantify the enantiomers of methamphetamine and amphetamine in urine by sheathless capillary electrophoresis-mass spectrometry with electrospray ionization.

Methods: Reagent grade chemicals were purchased on-line from VWR International. HS-γ-CD, 20% solution in water, was obtained from Beckman Coulter Inc., Brea, CA, USA. Drug standards were purchased from Cerilliant Corporation, Round Rock, TX, USA. Stock solutions were prepared at a concentration of 1 mg/mL in methanol and were further diluted for spiking urine samples. Standard solutions for mass spectrometry and extractions were prepared at 1 ng/µL in 5 to 50 mM Ammonium Formate (pH 2.85). Urine samples from a volunteer were spiked at 50 ng/mL and diluted with blank urine to 5 and 0.5 ng/mL, for preparation of three point HS-γ-CD calibrations. The samples were kept frozen until the time of analysis. Spiked urine samples and blanks were prepared by liquid-liquid extraction.

Results: The results show a well resolved separation of the enantiomers of methamphetamine and amphetamine ($R > 2.8$) in less than 15 minutes. The enantiomers were separated and quantified in urine extracts over three orders of magnitude 0.5 to 50 ng/mL of urine. The LOD was 0.5 ng/mL, the low calibrator.

Conclusion: The separation and sensitivity of the analysis provides a fast and effective method for confirmation and quantification of the enantiomers of methamphetamine and amphetamine in forensic cases.

References: 1. Rudaz, S., et al "Rapid stereoselective separations of amphetamine derivatives with highly sufated cyclodextrin", Electrophoresis 2005, 26 3910-3920.

Keywords: Methamphetamine, Amphetamine, Sheathless CE-MS

S46
Detection of Beta-Keto Amphetamines in Urine by GC/MS

Kayla Ellefsen*, Sarah Kerrigan; Sam Houston State University, Huntsville, Texas, USA

Introduction: Designer drugs have posed a challenge for law enforcement and forensic scientists for decades, but more recently the proliferation of analogs has overwhelmed the scientific community. Beta-keto amphetamine (BKA) drugs are the most recent family of psychedelic phenethylamines to pose a toxicological concern. The majority of BKAs are derived from cathinone, the active ingredient of Khat (*Catha edulis*), a plant native to Africa and the Middle East. These drugs are capable of producing powerful stimulant and hallucinogenic effects. Many have been associated with impaired driving and there have been several fatal intoxications.

Objective: The aim of this study is to develop an assay capable of detecting eight of the BKAs in urine by gas chromatography- mass spectrometry (GC/MS), using a solid phase extraction procedure (SPE) and evaluate the potential of a novel derivative to improve the analytical characteristics of these drugs.

Methods: Gas chromatography-mass spectrometry (GC/MS) and solid phase extraction was used to isolate and identify target drugs in urine. These included mephedrone (4-methylmethcathinone), methedrone (4-methoxy-*N*-methylcathinone), methylone (3, 4-methylenedioxy-*N*-methcathinone), butylone (beta-keto-*N*-methylbenzodioxolylpropylamine), ethylone (3,4-methylenedioxy-*N*-ethylcathinone), MDPV (3,4-methylenedioxypyrovalerone), naphyrone (napthylprovalerone), and 4-fluoromethcathinone.

Underivatized and derivatized extracts were investigated. 2, 2, 2-Trichloroethyl chloroformate (TCEC) was evaluated as a potential derivative and a preliminary evaluation of assay performance in urine showed it to be effective for qualitative analysis. Methylenedioxyamphetamine-(MDA-d5) was used as the internal standard and samples were acquired using synchronous SIM/SCAN. The susceptibility to evaporation and the need to salt out extracts to reduce volatility was also investigated. An existing stimulant SPE procedure was optimized for the BKAs and assay performance was evaluated.

Results: Rationalization of spectra indicated characteristic losses for the beta-keto amphetamines that limit the quality of the acquired data. SIM/SCAN acquisition of the TCEC derivatized extracts produced detection limits of 5 ng/mL for mephedrone, methylone, butylone, MDPV, naphyrone and 4-fluoromethcathinone; 10 ng/mL for methedrone and ethylone. Optimum results were obtained using a Polychrom Clin II column with an elution solvent consisting of methylene chloride/isopropyl alcohol (95:5) containing 2% concentrated ammonium hydroxide. All extracts were salted out prior to evaporation to minimize sample loss. Interferences from common drugs of abuse and other structurally related compounds were investigated. Although the assay was developed for qualitative use only, precision, accuracy and limits of quantitation were investigated in order to evaluate the limits of the assay. The most significant limitation was reproducibility. Replicate analysis of a urine sample containing 50 ng/mL of each analyte produced CVs of 8.6-17.7% (n=6). CVs were consistently higher than optimal over a range of concentrations. Accuracy over the same range was 107-117% (n=6). Limitations of the assay relate to the need to acquire full scan data for analytes that do not have secondary or tertiary ions of significant intensity to facilitate reliable analysis by SIM alone.

Conclusions: A qualitative procedure for the analysis of common beta-keto amphetamines in urine is described. Although the procedure lacked the reproducibility necessary for quantitative analysis, SIM/SCAN acquisition proved highly effective, facilitating the detection of low ng/mL concentrations using GC/MS.

Keywords: Designer Drugs, Cathinones, Derivatization, Urine, Gas Chromatography-Mass Spectrometry

Abstracts Of Poster Presentations

P01
Abstract Withdrawn

P02

Identification of Volatiles by Headspace Gas Chromatography with Simultaneous Flame Ionization and Mass Spectrometric Detection

Nicholas B. Tiscione*, Ilene Alford, Dustin Tate Yeatman, Xiaoqin Shan; Palm Beach County Sheriff's Office, West Palm Beach, FL, USA

Introduction: Volatiles are commonly abused as inhalants. The methods used for identification are generally non-specific if analyzed concurrently with ethanol or require an additional analytical procedure that employes mass spectrometry.

Objective: A novel technique utilizing a Dean's Switch style splitter to simultaneously quantitate and confirm ethyl alcohol and identify inhalants by flame ionization (FID) and mass spectrometric (MS) detection after headspace sampling and gas chromatographic separation is presented.

Methods: Using 100 µL of sample the limit of detection (LOD) and limit of quantitation (LOQ) was 0.005 and 0.010 g/dL, respectively for ethanol. The zero-order linear range ($r2 > 0.990$) was determined to span the concentrations of 0.010 to 1.000 g/dL. The coefficient of variation (CV) of replicate analyses was less than 3.1%. Quantitative accuracy was within ± 8%, ± 6%, ± 3%, and ± 1.5% at concentrations of 0.010, 0.025, 0.080, and 0.300 g/dL, respectively. In addition, methanol, acetaldehyde, isopropanol, acetone, toluene, isoamyl alcohol, isobutyl alcohol, n-butyl alcohol, 1,1-difluoroethane (DFE), 1,1,1,2-tetrafluoroethane (HFC-134a), chloroethane, trichlorofluoromethane (Freon®-11), dichlorodifluoromethane (Freon®-12), dichlorofluoromethane (Freon®-21), chlorodifluoromethane (Freon®-22), and 1,2-dichlorotetrafluoroethane (Freon®-114) were validated for qualitative identification by this method. The validation for qualitative identification included evaluation of matrix effects, sensitivity, carryover, specificity, repeatability and ruggedness / robustness.

Results: In a two-year period, DFE was identified by this method in one urine and seven whole blood antemortem driving under the influence case samples. Toluene was identified in one whole blood antemortem driving under the influence case sample in this same time period.

Conclusion/Discussion: The validated FID/MS method provides a robust procedure for the quantitation of ethyl alcohol in blood by FID with simultaneous confirmation by MS and can also be utilized as an identification method for inhalants such as 1,1-difluoroethane and toluene in blood and urine samples.

Keywords: Volatiles, Inhalants, GC-FID/MS

P03

Analysis of Amphetamine(s) and Bathsalt Type Drugs in Hair Using SPE and LC-MS/MS

Jeffery Hackett*[1], Albert A. Elian[2], Kerrie T. Donovan[2], and Michael J. Telepchak[1];
[1]UCT Inc., Bristol, PA, USA, [2]Massachusetts Police State Crime Laboratory, Sudbury, MA, USA

Introduction: In this presentation, attendees will learn about the analysis of Amphetamine(s) and bathsalt type drugs in hair using readily available solid phase extraction (SPE) cartridges and tandem mass spectrometry. Use of this method will permit analysts to provide data on these compounds in hair samples.

Objective: To provide analysts in the areas of forensic hair testing with a robust, reliable method for routine analysis of amphetamines and bathsalt type drugs in small samples of hair.

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. Samples of decontaminated hair (10 mg) were digested in 1 M NaOH (containing deuterated analogues) for 1 hour at 70 ºC. The samples were cooled, and glacial acetic acid (100 µL) was added. Each solution was adjusted to pH 6 with 0.1 M phosphate buffer (4 mL) and applied to a conditioned SPE column. After loading the sample, the sorbent was washed with deionized water, acetic acid (0.1 M), and methanol (3 mL of each, respectively). Each SPE column was dried and eluted with 3 mL of a solvent consisting of methylene chloride/isopropanol/ ammonium hydroxide (78:20:2). After elution, 200 µL of mobile phase was added to the collection tube. The samples were then evaporated to the mobile phase for analysis by LC-MS/MS in positive multiple reaction monitoring (MRM) mode. Data is presented for MRM's of amphetamine and bathsalts analogues:(amphetamine, methamphetamine, MDA, MDMA, butylone, ethylone, flephedrone,methylone, methedrone,mephedrone, MDPV, and pyravalerone).Liquid chromatography was performed in gradient mode employing a 50 x 2.1 mm biphenyl analytical column and a mobile phase consisting of acetontitrile and 0.1% aqueous formic acid. Chromatograms (presented) show efficiency of the method.

Results: The limits of detection/ quantification for this method were determined to be 0.05 ng/ mg and 0.1 ng/ mg, respectively for amphetamine/bathsalts analogues. The method was found to be linear from 0.1 ng/ mg to 10 ng/ mg (r^2>0.999). Data is presented to show that recoveries were found to be greater than 95% for all the amphetamine/bathsalt analogues. Interday and Intraday analysis of the amphetamine/bathsalt analogues were found to < 8% and < 12 %, respectively. Matrix effects were determined to be < 6% for the amphetamine/bathsalt analogues. Concentrations of amphetamine in real samples of hair were found to range from 1.2ng/ mg to 1.3 ng/ mg. Other amphetamine/ bathsalt analogues were not found to be present in the hair samples.

Discussion: Amphetamines and the newer bathsalt type drugs are challenging drugs for the forensic community. Hair is a matrix that is coming into its own as a forensic sample of choice after blood and urine. A method of analysis that can determine the presence of these drugs in samples of hair only be of a greater benefit for the forensic community at large. This methodology has demonstrated that not only can these drugs be analyzed, but quantified too.

Conclusion: The use of this new procedure for the analysis of amphetamine/bathsalt related compounds will be of great use to analysts in the field of forensic hair analysis as it demonstrates the use of SPE/LC-MS/MS to provide data from small amounts of hair sample.

Keywords: Hair, LC-MS/MS, SPE

P04
Bath Salts : Observations And Results From Eight Cases

C. Clinton Frazee III*[1], Uttam Garg[1], Diane C. Peterson[2], Robert Pietak[2], Mary Dudley[2] ; [1]Department of Pathology and Laboratory Medicine, Children's Mercy Hospitals and Clinics, Kansas City, MO, USA, [2]Office of the Jackson County Medical Examiner, Kansas City, MO, USA

Introduction: New illicit drugs sold deceptively as "bath salts" have gained widespread attention around the U.S. and, thus, necessitate that clinical and forensic toxicology laboratories include these drugs in their routine drug screens. Four active and commonly detected constituents in "bath salts" include 3,4-methylenedioxypyrovalerone (MDPV), methylone, mephedrone and methedrone. Through self-reporting, police observations and/or health care professional's reports in literature, a number of clinical symptoms have been reported for those who ingest or snort these "bath salts". These symptoms mirror the symptoms observed in methamphetamine and ecstasy abuse and include hypertension, tachycardia, vasoconstriction, hallucinations, psychosis, aggression, seizures and panic attack. Despite legislative efforts to ban the sale of these drugs, "bath salts" have maintained a presence in the illicit drug market.

Objectives: Our laboratory detected the presence of "bath salts" in two postmortem cases, three clinical pediatric cases, and three clinical referral cases in 2011. We present the results and/or symptoms of two unrelated fatalities and three clinical pediatric emergency room admissions involving "bath salts" that were received by our laboratory in 2011.

Methods: A liquid-liquid alkaline extraction followed by chromatography/mass spectrometry (GC/MS) scan analysis was used to detect the "bath salt" constituents in each case.
Reference standards were purchased from Cerilliant Corporation and used to qualitatively identify any bath salts presence. Quantitations were performed by external laboratories.

Results: Mephedrone and methedrone were not detected in any of the samples. In all cases, methylone and MDPV were the only two "bath salt" constituents detected by our laboratory. In the first fatality, MDPV, tramadol, alprazolam and diphenhydramine were detected. The concentrations of MDPV, alprazolam and tramadol were 130 ng/mL, 26 ng/mL and 9000 ng/mL, respectively. Diphenhydramine was not quantitated. In the second fatality, methylone and lamotrigine were detected at concentrations of 3300 ng/mL and 2.5 µg/mL respectively. In each of the three clinical pediatric cases, methylone was detected in combination with other illicit and/or prescription drugs.

Conclusion/Discussion: In the first fatality, the cause of death was concluded to be tramadol overdose with MDPV as a contributing factor, and the manner of death was accidental. In the second fatality, the cause of death was concluded to be acute methylone intoxication, and the manner of death was accidental. In the three clinical pediatric cases, the primary reported symptoms included seizures and loss of consciousness. Because "bath salts" can cause significant morbidity and mortality, especially when used in combination with other prescription and/or illicit drugs, it is important for clinical and forensic toxicology laboratories to maintain an awareness of the "bath salt" symptomatology and include these drugs in their list of detectable compounds.

Keywords: Bath Salts, Methylone, MDPV

P05
Driving Under the Influence of Foxy and Marijuana - A Case Report

Xiaoqin Shan*, Nicholas B. Tiscione, Ilene Alford, and Dustin Tate Yeatman; Palm Beach County Sheriff's Office, West Palm Beach, FL, USA

Introduction: Foxy or foxy methoxy is the street name for 5-methoxy-N, N-diisopropyltryptamine (5-MeO-DIPT). It is a tryptamine derivative that possesses hallucinogenic/stimulant properties, and hence, became a designer drug used as a substitute for ecstasy. Although 5-MeO-DIPT can cause impairment that affects the ability to operate a motor vehicle safely, published reports of driving under the influence of this substance showing levels in blood are rare, if any.

Objective: To present a driving under the influence (DUI) case involving 5-MeO-DIPT and marijuana.

Methods: A blood sample was collected two hours after the accident for toxicological analyses. Routine drug screening tests were performed by enzyme-linked immunosorbent assay (ELISA) for amphetamines, barbiturates, benzodiazepines, carisoprodol, cannabinoids, cocaine, methadone, and opioids and liquid-liquid extraction (LLE) with gas chromatography mass spectrometry (GC/MS) for other basic drugs including 5-MeO-DIPT. 5-MeO-DIPT analysis was validated for quantitation by gas chromatography with nitrogen phosphorus detection (GC/NPD) using PCP-d5 as the internal standard and confirmation by GC/MS. Analysis for delta-9 tetrahydrocannabinol (THC) and delta-9-carboxy tetrahydrocannabinol (THCA) was performed by solid phase extraction (SPE) with GC/MS in selective ion monitoring (SIM) mode.

Results: This case report describes a 23-year-old male driver who took what he believed to be ecstasy at a party before crashing into the back of an emergency-parked semi-trailer. At the accident scene the driver showed signs of impairment caused by stimulants and hallucinogens. He was observed lying on the ground screaming “I'm dead, I'm dead. I'm not going to wake up from this!" His pupils were dilated and his speech was very rapid with incomplete sentences. He admitted to the investigating police officer that he committed the traffic crash in a fit of rage and he was “tripping" on ecstasy. The screening tests showed that the blood sample was positive for cannabinoids and 5-MeO-DIPT and negative for alcohol and the rest of the panels. Quantitation analyses showed that 5-MeO-DIPT, THC, and THCA were present at 107 ng/mL, 5.6 ng/mL, and 23 ng/mL, respectively. The behavior of the driver was consistent with the stimulant and hallucinogenic effects of 5-MeO-DIPT combined with the effects contributed by THC.

Conclusion: We presented a DUI case involving 5-MeO-DIPT and THC. Either substance can cause impairment of cognitive and psychomotor functions, and in combination, may lead to significant impairing effects on drivers.

Keywords: Foxy, Marijuana, DUI

P06

The Sale of Synthetic Marijuana and Bath Salts by a South Florida Smoke Shop - A Case Report

Amber C. Kohl, **Ilene K. Alford***, Palm Beach County Sheriff's Office, West Palm Beach, FL, USA

Introduction: The drugs detected by Forensic Chemistry labs give Toxicology labs insight as to drugs they may expect to see in biological samples. However, the drugs are often in significantly lower concentrations in blood and urine requiring sensitive testing methods. As many toxicology labs may not presently have methods in place to detect synthetic drugs, this case study presents a picture of the synthetic drugs that are currently on the market and may provide guidance in the selection of target analytes for method validation.

Objective: In the summer of 2011, the owner and an employee of a smoke shop in West Palm Beach, Florida, were arrested for the suspected sale of controlled substances after a 3-month undercover investigation by the Palm Beach County Sheriff's Office. The shop was openly selling products packaged as powders, pills and potpourri with names such as Bliss, Space Trips, and Jazz Blueberry, respectively. There was also a house blend smoking product kept behind the counter that was only sold to customers requesting it. This case report reveals current trends in the drugs that are being marketed as Spice and bath salts for a legal alternative to marijuana and stimulants.

Methods: The submitted samples were initially weighed and spot tested. Then a portion of the sample was dissolved in an aminopyrine methanolic internal standard and analyzed by GC and GC-MS.

Results: The following drugs were identified and/or confirmed in the various powder, pill and plant submissions: JWH-018, JWH-122, JWH-203, AM-2201, N-ethylamphetamine, naphyrone, alpha-pyrrolidinopropiophenone (PPP), methylone and 4-methylethcathinone. The controlled substances at the time of analysis were N-ethylamphetamine, JWH-018, methylone and 4-methylethcathinone. Over 20 additional non-controlled substances were also detected in an initial screen by GC-MS, but were not confirmed due to standard material availability. In addition, phenazepam was also identified in a beige triple scored tablet advertised as a “Zaney Bar".

Conclusion/Discussion: The ongoing challenge with “synthetic marijuana" and “bath salts", as with all synthetic designer drugs, is the rapid modification to new analogues that are not included on controlled substance lists in order to circumvent drug laws. Chemically modified stimulants and cannabinoids are also often marketed as bath salts and incense in an attempt to evade the law. The packages did not indicate the identity or dosage of any of the identified drugs. This presents a real danger to users, since the pharmacology and toxicology of most of these substances have yet to be fully examined. There has been a remarkable increase in synthetic drug abuse in recent years, signaling the need for toxicology labs to expand the scope of their testing to include synthetic cannabinoids and stimulants.

Keywords: Synthetic Marijuana, Bath Salts

P07

Simultaneous Quantification of Amphetamines and Opiates in Human Hair by Liquid Chromatography/Tandem Mass Spectrometry

Hsiu-Chuan Liu*[1], Ray H. Liu[2], Dong-Liang Lin[1]**;** [1]Department of Forensic Toxicology, Institute of Forensic Medicine, Ministry of Justice, Taipei, Taiwan, [2]Department of Medical Laboratory Science and Biotechnology, Fooyin University, Kaohsiung, Taiwan

Introduction and Objectives: Methamphetamine and heroin have historically been the most commonly abused drugs in Taiwan and are routinely monitored in our laboratory by gas chromatography-mass spectrometry methods. The purpose of this study was to evaluate whether the liquid chromatography-tandem mass spectrometry (LC-MS/MS) based approach can be more effectively applied to the simultaneous quantitation of amphetamine (AM), methamphetamine (MA), morphine (MOR), codeine (COD), 6-acetylmorphine (6-AM) and 6-acetylcodeine (6-AC) in human hair specimens.

Methods: Hair samples were initially cut into 0.1-cm sections; washed with dichloromethane and sonicated in 2-mL methanol/trifluoroacetic acid (85:15; v/v) at 50 °C for 3 hours. The resulting solutions were processed with a solid-phase extraction procedure before undergoing LC-MS/MS analysis. Chromatographic separation was achieved using an Agilent Zorbax SB-Aq (100 mm x 2.1 mm i.d.; 1.8 µm particle) analytical column operated at 50 °C. The mobile phase consists of 0.1% formic acid (v/v) in water (A) and methanol (B). Mass spectrometric analysis was performed in positive-ion mode; applying multiple reaction monitoring (MRM) using optimized collision energy for each precursor ion designating each analyte of interest.

Results: The overall protocol achieved the following results when applied to the analysis of 50 mg drug-free hair specimens fortified with 100-10000 pg/mg analytes of interest: (a) inter-day and intra-day precision ranges (percent CV) for AM, MA, MOR, COD, 6-AM and 6-AC were 1.0-5.1%, 1.9-5.6%, 1.0-7.4%, 1.0-7.6%, 2.2-4.0% and 1.3-8.2%, respectively; (b) method linearity (r^2); detection limit and quantitation limit for all 6 analytes were >0.998; 10 pg/mg and 50 pg/mg, respectively.

Conclusions: This protocol was then applied to (a) the analysis of hair samples collected from 77 self-reported drug abusers; and (b) to evaluate the deposition pattern of drugs in head hairs collected from four female methamphetamine and heroin abusers at a rehabilitation facility. We concluded that this relatively simple protocol can be used for routine and reliable identification and quantitation of AM, MA, MOR, COD, 6-AM and 6-AC in hair.

Keywords: Drugs of Abuse, Hair, LC-MS/MS

P08

Rapid Screening and Confirmation of 300 Pesticides in Postmortem Specimens by LC Ion-Trap MS/MS and Automated Library Search

Hsiu-Chuan Liu[1], Hsi-Tzu Lee[1], Chu-Chun Hsu[1], Ray H. Liu[2], **Dong-Liang Lin[*1]**; [1]Department of Forensic Toxicology, Institute of Forensic Medicine, Ministry of Justice, Taipei, Taiwan, [2]Department of Medical Laboratory Science and Biotechnology, Fooyin University, Kaohsiung, Taiwan

Introduction and Objectives: Preliminary screen of drugs and toxic compounds is a challenge and plays an important role in forensic and clinical laboratories. Traditional methods for preliminary screen include immunoassay (IA), gas chromatography-nitrogen/phosphorous detection (GC/NPD), mass spectrometry (MS), GC/MS, and liquid chromatography-diode array detection (LC/DAD). Recent advances in the LC-MS/MS technology have provided an opportunity for the development of more specific approaches to achieve the "screen" and "confirmation" goals in a single analytical step. The objective of this study is to establish a 300 pesticides MS/MS library by LC ion-trap MS/MS instrumentation, using a linear ramping collision energy technique, for general unknown screening (GUS) application in forensic and clinical laboratories.

Methods: Liquid-liquid extraction procedure using Toxi-tubes® A protocol was coupled to LC-ESI-MS/MS (Agilent 6320 LC/MSD trap) using an Agilent Zorbax SB-Aq (2.1 mm x 100 mm, 1.8 μm particle) analytical column operated at 50°C. The analytes were eluted at a flow rate of 0.32 mL/min with a solvent mixture composed of methanol and water containing 0.1% formic acid. Positive-ion ESI MS/MS spectra and retention time for each of the pesticide was first established using 1000 ng/mL standards. These spectra were then transferred to the library and searched by the identification algorithm of the Agilent ion trap software for matching the spectra derived from unknown compounds found in the test specimen. Scores for Fit, Reverse Fit, Purity and Retention time are provided for each match.

Result: The analysis of the sample is carried out using the “AutoMS(2)” mode in which the signal is monitored in full-scan. When the intensity of an ion within the selected window exceeds a minimum threshold value, that ion is then selected as a precursor ion and fragmented. Up to five precursor ions in each MS spectrum may be selected. Each precursor ion can be selected for two occurrences and then excluded for 0.2 min. Since the resulting data file consists of MS and MS/MS spectra, a special algorithm in the software is used to generate the MS/MS spectrum pair (for each compound) that are related by a common MS precursor ion. Molecular weight and retention time are provided for each spectrum match. In this study, each pesticide has its specific retention time, the retention time window for each component is set at ±1 min. The time window is important because if another compound elutes later also includes the same *m/z* precursor ion, it will not be confused with the earlier eluting one.

Conclusions: This method provides a rapid, sensitive approach to isolate, screen and confirm a broad spectrum of pesticides. The specificity of the method was evaluated using numerous postmortem matrices. No significant interference was found at the retention time expected of the targeted compounds. This method was successfully applied to the analysis of postmortem specimens from forensic cases.

Keywords: LC-MS/MS, Library Search, Pesticides

P09
Distribution of Chloralose in a Fatal Intoxication

Alberto Salomone*[1], Enrico Gerace[1], Valentina Ciccotelli[2], Paola Rapetti[3], Marco Vincenti[4];
[1]Centro Regionale Antidoping "A. Bertinaria", Orbassano, Turin, Italy, [2]Istituto Zooprofilattico del Piemonte Liguria e Valle D'Aosta, Turin, Italy, [3]S.C. Medicina Legale, Turin, Italy, [4]Dipartimento di Chimica, Università di Torino, Turin, Italy

Introduction: Chloralose (alpha-chloralose) is a poisonous substance currently used as a rodenticide or avicide. It has used primarily in Europe since 1893 as a human and veterinary hypnotic agent. Chloralose is a central nervous system depressant also acting as a stimulant on spinal reflexes. In the present case, a 24-year-old man was found dead in his bedroom near vomit residues. Several items were seized from the scene, including an empty bottle of Murex® 50 g (α-chloralose), sold in Italy as rodenticide. Postmortem examination revealed no evidence of natural disease or trauma.

Objective: Our laboratory was asked to execute the inherent toxicological analyses in order to determine if a possible massive drug consumption could be taken into account as the cause of the death. The specimens sampled during the autopsy included heart blood, urine, vitreous humour, gastric contents, brain, bile and liver.

Methods: Several extraction procedures and a specific LC-MS/MS protocol were purposely developed and validated. Chromatographic separation was performed using an Agilent 1100 series liquid chromatographer and detection was carried out by an Applied Biosystems API 3200 triple quadrupole mass spectrometer.

Results: The method was validated by investigating the following parameters: selectivity, linearity, identification and quantitation limits (LOD and LOQ), precision, accuracy and recovery. Matrix effects phenomena were also evaluated. Chloralose was found in blood at a concentration of 65.1 mg/L and high levels were also detected in the gastric contents, confirming its ingestion shortly before the man's death. The distribution of chloralose in the body was evaluated by analyzing urine, vitreous humour, brain, bile and liver specimens.

Conclusions: This report presents a fatal case involving acute intoxication by alpha-chloralose. A specific LC-MS/MS procedure was developed and validated in order to specifically and accurately detect the drug in various human fluids and tissues. The high concentration in the gastric content proved the recent massive ingestion of chloralose. Chloralose was measured in bile, liver, brain and vitreous humour showing a significant presence of the drug. On the other hand, vitreous humour was not a major site of alpha-chloralose deposition as previously described in fatal cases involving an acute consumption of chloralose. Lastly, the heart blood concentration was in the range considered as highly toxic, although lower than that found in previous cases. In conclusion, the agreement between the postmortem examination and the toxicological findings were consistent with fatal chloralose intoxication.

Keywords: Chloralose, Rodenticide, Suicide, LC-MS/MS

P10

Application of Ultra High Pressure Liquid Chromatography Tandem Mass Spectrometry to the Analysis of Classical Antipsychotic Drugs in Serum and Plasma

JoEtta M. Juenke[1], **Kendall A. Miller***[1], Paul I. Brown[1], Francis M. Urry[1], Kamisha L. Johnson -Davis[1,2]; [1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, USA, [2]Department of Pathology, University of Utah, School of Medicine, Salt Lake City, UT, USA

Background: Classical antipsychotic drugs are commonly prescribed in the USA and are monitored by liquid and gas chromatographic methods. Runtimes, interferences, and extractions for liquid chromatographic methods have been improved using ultra high pressure liquid chromatography-tandem mass spectrometry. A rapid and simple procedure for monitoring haloperidol, thiothixene, fluphenazine, and perphenazine was developed using a Waters Acquity system.

Method: Antipsychotic drug concentrations in serum and plasma were determined by ultra pressure high performance chromatography with tandem mass spectrometry detection (Waters Acquity UPLC TQD; [UPLC-MS/MS]). The instrument is operated with an ESI interface; in multiple reaction monitoring (MRM); and positive ion mode. The resolution of both quadrupoles was maintained at unit mass resolution with a peak width at half height of 0.7 amu. The data analysis is performed using the Water Quanlynx software. Serum samples were thawed at room temperature and a 100-µL aliquot was placed in a tube. Then 300-µL of precipitating reagent (acetonitrile-methanol [50:50; volume: volume]) containing the internal standard (0.12 ng/µL Imipramine-D3) was added to each tube. The samples were then vortexed and centrifuged. The supernatant was transferred to an autosampler vial and 8-µL was injected into the UPLC-MS/MS. Utilizing a Waters Acquity UPLC HSS T3 1.8 µm, 2.1 x 50mm column at 25ºC the analytes were separated using a timed gradient using a linear gradient of acetonitrile and water; each having 0.1% formic acid added. The column is eluted into a Waters Acquity UPLC TQD; operating in a positive mode to detect Imipramine D3 at transition 284.25>89.10; haloperidol at 376.18>165.06; thiothixene at 444.27>139.24; fluphenazine at 438.27>171.11; and perphenazine at 404.19>143.07. Secondary transitions for each analyte are also monitored for Imipramine D3 at transition 284.25>193.10; haloperidol at 376.18>122.97; thiothixene at 444.27>97.93; fluphenazine at 438.27>143.08; and perphenazine at 404.19>171.11. The run-time is 1.8 minutes per injection with baseline resolved chromatographic separation.

Results: The analytical measurement range was 0.2 to 12.0 ng/mL for fluphenazine and perphenazine. The analytical measurement range for haloperidol and thiothixene was 1 to 60.0 ng/mL. Intra-assay imprecision (CV) and inter-assay imprecision (CV) was less than 15% at two concentrations for each analyte.

Conclusions: By utilizing a UPLC-MS/MS method we combined three analytical assays into one; which reduced the time for sample preparation and set-up, analytical run-time, and turn-around time for analysis. Implementing UPLC had eliminated chromatographic interference issues with our previous HPLC method.

Keywords: Tandem Mass Spectrometry, Anti-Psychotic, Therapeutic Drug Monitoring

P11
Degradation of Bupropion in Postmortem Specimens

Erin M. McIntee*, Marc S. Feaster, Sandra C. Bishop-Freeman, Ruth E. Winecker; Office of the Chief Medical Examiner, Chapel Hill, NC, USA

Introduction: Bupropion is used in the treatment of depression (Wellbutrin®) and smoking cessation (Zyban®). It is seen regularly in postmortem cases including fatalities due to bupropion overdose. Early research found that bupropion degraded substantially when analyzed in formaldehyde and plasma. In our laboratory, a significant reduction of bupropion with respect to its metabolite, threo-bupropion, was observed between screening and quantification to merit investigation.

Objective: In this study bupropion and its metabolite threo-bupropion were monitored over a 50-day period to observe the degradation of bupropion in blood, liver and liver homogenate. In order to simulate various forensically relevant circumstances; aliquots of the samples were stored at different temperatures.

Method: Previously identified postmortem blood and liver samples containing elevated levels (>1.0 mg/L) of bupropion were aliquoted to establish three groups that were stored at room temperature (RT), in the refrigerator (RF) and in the freezer (FR). The samples were extracted with a basic liquid-liquid procedure and analyzed on a gas chromatograph with a nitrogen/phosphorous detector. The blood and liver samples were analyzed on day 0, 15, 29 and 50. In addition, the liver samples were analyzed on day 7.

Results: Blood, liver and liver homogenate samples stored at room temperature were found to have a drastically reduced concentration of bupropion in comparison to the concentrations found in the samples stored in the refrigerator and freezer. In the blood samples, the concentrations were less than the limit of quantification at Day 15, the liver and liver homogenate samples had a similar result by Day 30. The samples stored in the refrigerator had less degradation in comparison to those stored at room temperature. Those stored in a freezer had the least amount of degradation if any. Meanwhile, the concentration of threo bupropion deviated little from the initial analysis. This supports the theory that bupropion is not being converted to threo bupropion.

Conclusions: According to data collected in this study, tissue samples from a suspected bupropion case should be stored in the freezer and blood should be refrigerated or, if possible, frozen promptly after collection in order to prevent bupropion degradation. A suspected bupropion overdose case should be interpreted with caution if the decedent was not discovered quickly after death. Alternatively, as the threo-bupropion metabolite appears to be stable, diagnosis of bupropion overdose may be possible via interpretation of the threo-bupropion concentration. Finally, autopsy samples not recovered and properly stored promptly after a bupropion death will be subject to degradation of the parent compound.

Keywords: Bupropion, Degradation, Postmortem

P12

A Rapid Online Extraction Method for the Quantitation of Leflunomide Metabolite With a Novel Mathematic Approach to Competitive Ion Suppression

Jessica Erbsen*[1], Matt Alstad[1], Loralie Langman[1], Christine Snozek[2]; [1]Toxicology and Drug Monitoring Laboratory, Mayo Clinic, Rochester, MN, USA, [2]Department of Lab Medicine and Pathology, Mayo Clinic, Scottsdale, AZ, USA

Background/Introduction: Leflunomide is a disease-modifying anti-rheumatic drug approved for therapy of rheumatoid arthritis and used off-label to reduce viral nephritis in renal transplant. It is a prodrug: rapid and complete metabolism converts leflunomide to its active metabolite, teriflunomide (also called A771726). This conversion is a simple ring-opening, making quantitation of either analyte by mass spectroscopy complex. Measurement of drug concentrations is performed both to monitor therapy and to assess drug clearance, thus a large analytical range is required.

Objective: The principle aim of this study was to develop a novel high-turbulence liquid chromatography tandem mass spectrometry (HTLC-MS/MS) method to measure teriflunomide.

Methods: Serum was diluted with aqueous internal standard (IS), extracted online with a Turboflow® Cyclone column, and separated on a BDS Hypersil C8 analytical column. Analysis was on an ABSciex 3200 LC-MS/MS in negative ion mode with electrospray ionization, monitoring m/z 269.0/82.0 and 269.0/160.0 for teriflunomide and m/z 273.0/82.0 and 273.0/164.0 for teriflunomide-d4. Total analysis time was 3 minutes per sample using the multiplexing system.

Results: The method was validated over two calibration curves, from 0.020 - 0.5 µg/mL for drug clearance and 0.5 - 100 µg/mL for monitoring therapy, for a dynamic range of 0.020 - 100 µg/mL. Intra- and inter-day imprecisions across the entire range were <5% and <7%, respectively. Accuracy was assessed by comparison with an outside laboratory, showing a mean difference of <10% (range -0.6% to 20.9%). Linear regression yielded a slope of 0.994 and correlation coefficient of 0.9996. No analytical interferences were found amongst 53 commonly prescribed and abused drugs, including parent leflunomide. Competitive ion suppression between the analyte and the IS was observed, where increasing amounts of the unlabeled drug were associated with reduced response of the labeled IS. A novel mathematical tool was developed to monitor IS response to detect sample-specific ion suppression.

Conclusion/Discussion: We validated a rapid and robust analytical method for the quantitation of teriflunomide by HTLC-MS/MS. No other published methods have a run time as rapid or utilize online extraction. Furthermore, we report a novel tool exploiting the mathematical relationship between analyte concentration and IS response that accommodates the problem of competitive ion suppression. The IS area is frequently used as a marker for ion suppression, extraction efficiencies, spiking errors, and interferences. We discovered that the IS response decreased in a consistent fashion with increasing concentrations of unlabeled drug. This suggested that teriflunomide and its labeled IS experienced mutual, competitive ion suppression in the mass spectrometer source. This logarithmic relationship was used to calculate an expected IS response based on the concentration of unlabeled teriflunomide in calibration standards. The percent difference of the expected and observed IS response was monitored in patient samples and quality controls to detect outliers for each run. Competitive in-source ion suppression is a growing concern for clinical laboratories that are under regulatory guidelines to track IS responses. The simple mathematical tool reported here overcomes the problem of competitive ion suppression to permit accurate monitoring of IS response in patient samples.

Keywords: Leflunomide, LC-MS/MS, Ion Suppression

P13

Development of a Highly Specific Polyclonal Antibody for the Determination of Venlafaxine and its Major Active Metabolite O-Desmethylvenlafaxine

M. Cleland, A-E. Brogan, **M.E.O. Benchikh***, R.I. McConnell, S.P. Fitzgerald; Randox Toxicology Limited, Crumlin, UK

Background: Venlafaxine is one of a new class of anti-depressant medications that affect neurotransmitters such as serotonin; dopamine and norepinephrine within the brain. An imbalance in these neurotransmitters is believed to be the cause of depression and may also play a role in anxiety. Venlafaxine is metabolised to O-desmethylvenlafaxine (major active metabolite) and two minor metabolites: N-desmethylvenlafaxine and O & N-di-desmethylvenlafaxine.

Objective: The aim of this study was to develop a highly specific polyclonal antibody for venlafaxine and its major active metabolite O-desmethylvenlafaxine. This antibody will facilitate the development of immunoassays for the detection of these compounds with applications to therapeutic drug monitoring (TDM) and toxicology.

Methods: Venlafaxine was conjugated to bovine thyroglobulin (BTG) as carrier. Adult sheep were immunized with the resulting immunogen on a monthly basis to provide target-specific polyclonal antisera. Immunoglobulin fraction was extracted and evaluated via competitive enzyme-linked immunosorbent assay (ELISA). The absorbance was read at 450nm.

Results: The developed polyclonal antibody was specific for venlafaxine hydrochloride (%cross-reactivity 100%) and the %cross-reactivity with the major metabolite O-desmethylvenlafaxine was 48%. The antibody did not cross-react with tramadol; N-desmethyltramadol; O-desmethyltramadol; meperidine; methylphenidate and phencyclidine (PCP). The sensitivity, expressed as half maximal inhibitory concentration (IC_{50}) was 18.31ng/ml (venlafaxine hydrochloride as calibrator; range 0-320ng/ml).

Conclusion: The results indicate that the developed polyclonal antibody is specific for venlafaxine and its major active metabolite O-desmethylvenlafaxine. This antibody is suitable for development of immunoassays for the determination of these compounds in TDMs and toxicology applications.

Keywords: Venlafaxine, Anti-Depressants, Antibody, Immunoassays

P14
Development of a Generic Polyclonal Antibody to 'Bath Salts'

J.Taggart, S. Savage, **M.E.O. Benchikh***, R.I. McConnell, S.P. Fitzgerald, Randox Toxicology Limited, Crumlin, UK

Background: 'Bath Salts' are new additions to the designer drugs group, first reported in the US in late 2009. They typically contain methylenedioxypyrovalerone (MDPV), mephedrone and/or naphyrone; which act as central nervous system stimulants in a similar manner to methamphetamine, ecstasy and cocaine. Abuse of these substances can cause adverse side effects, such as increased heart rate, increased blood pressure, insomnia, nausea, vomiting, hallucinations, paranoia and anxiety. Numerous deaths associated with the use of 'Bath Salts' have been reported in several countries, including USA, UK and Switzerland. These drugs are still sold as 'legal highs'.

Objective: The goal of this study was to develop the first generic polyclonal antibody against 'Bath Salts'; namely MDPV, 3',4'-methylenedioxy-alpha-pyrrolidinobutiophenone (MDPBP), 4'-methyl-alpha-pyrrolidinobutiophenone (MPBP) and naphyrone. This antibody could be used for further development of immunoassays for forensic and toxicological applications.

Method: MDPBP hapten was synthetized and conjugated to bovine thyroglobulin (BTG), an immunogenic carrier protein. The resulting immunogen was administered to adult sheep on a monthly basis to generate target-specific polyclonal antiserum. Immunoglobulin fraction was extracted from the antiserum and evaluated via competitive biochip immunoassay.

Results: The developed polyclonal antibody presented the following specificity profile (expressed as %cross-reactivity): 100% (MDPBP), 75% (MDPV), 21% (naphyrone), 12% (MPBP), <5% methylenedioxypyrovalerone (MDPP). No cross-reactivity with amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), mescaline and (+)-pseudoephedrine was observed. Displacement of maximal signal following addition of MDPBP at various levels ranging from 0 to 1053ng/ml was calculated. The antibody exhibited a half maximal inhibitory concentration (IC_{50}) of 30.7ng/ml with MDPBP.

Conclusion: Results indicate that the developed antibody detects: MDPBP, MDPV, naphyrone; MPBP, MDPP. This antibody can be used for the development of immunoassays for the detections of 'Bath Salts' for forensic and toxicological applications.

Keywords: 'Bath Salts', MDPV, Synthetic Cathinones, MDPBP, Methylenedioxypyrrolidinocathinones

P15

Positive CEDIA® Drugs of Abuse Assay Results Arising From New Psychoactive Substances

Siobhan Stokes*[1], Sinead McNamara[1], Seibh Conroy[1], Ian Walsh[1], Pierce Kavanagh[2]; [1]Drug Treatment Centre Board, Dublin, Ireland, [2]Department of Pharmacology and Therapeutics, School of Medicine, Trinity College Dublin (TCD), Centre for Health Sciences, St. James's Hospital, Dublin, Ireland

Background: The use of new psychoactive substances known variously as Head Shop/Party pills/Bath salts has been an issue over recent years in many countries. In 2010, a record 41 new substances were reported to the European early warning mechanism. Preliminarily data for 2011 shows no sign of decline. In 2011 a study by LCMS of false positive rates in samples tested for drugs of abuse in our laboratory showed that 22% of samples testing positive with the CEDIA Amphetamine/Ecstasy assay contained instead other psychoactive substances including 2-aminoindane, mephedrone, methylone, 3-fluoromethcathinone, methedrone , 4-methylethcathinone, dimethocaine, phenethylamine, BZP and 3-TFMPP. Many samples contained multiple drugs. Evidently levels of some of these drugs or their metabolites in urine can be high enough to trigger positive results on the CEDIA Amphetamine/Ecstasy assay. Investigation of cross-reactivity is needed to identify which substances may generate positive results on routine drugs of abuse screening tests. Due to their novelty, many new drugs have not yet been tested by the immunoassay manufacturer.

Objective: Evaluation of individual cross-reactivity of 56 drugs including many new psychoactive substances on CEDIA routine drugs of abuse assays.

Methods: The CEDIA kits were set up according to manufacturer's instructions for qualitative screening on Beckman Coulter AU2700 chemistry analysers. Reagents, quality controls and calibrators were supplied by Microgenics. Standards were purchased from LGC where available or provided by Dr Pierce Kavanagh[2]. Urine spikes fortified at a level of 100,000ng/ml with 56 drugs were analysied using Amphetamine/Ecstasy, Cocaine, Opiate, Methadone Metabolite (EDDP), Benzodiazepines, Multi-level THC and Heroin Metabolite (6-AM) CEDIA assays. The assays are normalised to give a reading of 100 at the cut-off for each assay. Any reading in excess of 100 is positive.

Results: Amphetamine/Ecstasy: 23 drugs gave **positive** readings. N-methyl-5-APB and 5-APB gave readings . **500**. 4-methylmethamphetamine, DMAA and 3-methiopropamine gave readings between **300**-**500**. 2-methiopropamine, butylone, phenethylamine, pentylone, 3-TFMPP, camfetamine, m-CPP and buproprion gave readings between **200**-**300**. Dimethocaine, β-ethylmethcathinone hydrochloride, 4-EMC, buphedrone, MDAT, pFPP, 5-IAI, BZP, 3,4-mehylenedioxy-N-benzylcathinone and MDPBP gave readings between **100**-**200**. Cocaine: Fluorotropacocaine, (+)-4-Methyl-pyrrolidinopropiophenone and desoxypipradol gave readings > **100**. Heroin Metabolite (6-AM): MDPV and 4'-methyl-alpha-pyrrolidinobutiophenone hydrochloride gave readings of **99** and **84** respectively. Benzodiazepine: RCS-4 and AM694 gave readings of **77** and **70** respectively. Cannabis: Interestingly JWH018 and CP47-497 and AM694 showed no significant cross reactivity.

Discussion: The CEDIA assays are qualitative with defined cut-offs. The linear range is unknown for these analytes so levels far lower than the 100,000ng/ml level spiked here may generate positive readings. For example bupropion and phenethylamine listed by the manufacturer as giving a positive reading for Amphetamine/Ecstasy assay at the 1000ng/ml cut-off at concentrations of 18,000ng/ml and 10,000ng/ml gave readings of 202 and 263 respectively.

Conclusion: We found that the Amphetamine/Ecstasy assay has cross reactivity to a significant number of new psychoactive drugs and related compounds. Some drugs also showed evidence of cross reactivity with cocaine, 6-acetylmorphine and benzodiazepine immunoassays.

Keywords: Immunoassay, Cross Reactivity, New Psychoactive Substances, Head Shop, Party Pills, Bath Salts

P16

Quantitative Chiral Determination of the d- and l- Enantiomers of Amphetamine and Methamphetamine in Oral Fluid Using HPLC/MS/MS

Michael Herrera*, David Kuntz, Clinical Reference Laboratory, Lenexa, KS, USA

Introduction: The testing of oral fluids for amphetamines requires that laboratories have the ability to test for the isomers of amphetamine and methamphetamine. Determination of the percent relative amounts of the d- and l- enantiomers for amphetamine and methamphetamine contributes further information that may be used to assess the source of the drug. A HPLC/MS/MS method for the chiral separation and quantitative determination of the d- and l- enantiomers for amphetamine and methamphetamine in oral fluid has been validated and applied to patient samples.

Objective: To validate a method for the chiral analysis of amphetamine and methamphetamine in oral fluids using HPLC/MS/MS and apply that method to the analysis of patient samples.

Methods: The oral fluid sample (0.2 mL) was treated with 10% sodium periodate for 30 minutes to oxidize sympathomimetic amines and was extracted using an Agilent SPEC-DAU 96 well SPE solid-phase extraction plate. The subsequent extract was dried and reconstituted with mobile phase. High performance liquid chromatographic (HPLC) chiral separation is performed on a Supelco Astec Chirobiotic® V2 column and detected using a Shimadzu Nexera UFLC system with an AB SCIEX API4000 triple-quadrupole mass spectrometer in the positive ion MS/MS mode. The mobile phase consisted of 99.89:0.1:0.01 methanol:acetic acid:ammonium hydroxide (v/v/v) at a flow rate of 0.5 mL/minute.

Results: The method is accurate and reproducible from 1 ng/mL to 200 ng/mL for each enantiomer. The intra-day (n=6 each day) and inter-day (n=18) reproducibility (CV) for all analytes is less than 6% across the linear range of the method. Preparation and quantitation of spiked 20% d-enantiomer controls (n=18 over three days) resulted in CV's of less than 2%. Patient oral fluid samples collected using the Intercept oral fluid collection device from Orasure Technologies, Inc.; previously determined to be negative for amphetamine and methamphetamine were spiked with these drugs and demonstrated no significant effect of buffer/matrix on analysis when compared to calibrator prepared in neat oral fluid. One-year-old oral fluid samples that were positive for amphetamine and/or methamphetamine were analyzed using this method. This method was found to provide accurate quantitative results compared to previously reported values and able to provide enantiomer identification. Amphetamine only positive samples were found to consist of approximately 70% of the d-enantiomer which is consistent with Adderall use. Methamphetamine positive samples were all nearly 100% d-enantiomer.

Conclusion: A quantitative chiral LC/MS/MS method has been validated for the determination of the *d*- and *l*-enantiomers of amphetamine and methamphetamine. The method is precise and accurate and is currently in use at Clinical Reference Laboratory for donor sample analysis.

Keywords: Amphetamines, Methamphetamine, Chiral, LC/MS/MS, Oral Fluid

P17

Positive CEDIA® Amphetamine/Ecstasy Assay Results Arising from New Psychoactive Substances

Sinead McNamara[1], **Siobhan Stokes*[1]**, Paul O'Byrne[1], Pierce Kavanagh[2]; [1]Drug Treatment Centre Board, Dublin, Ireland, [2]Department of Pharmacology and Therapeutics, School of Medicine, Trinity Centre for Health Sciences, St. James's Hospital, Dublin, Ireland

Background: In 2011, a study of false positive rates for drugs of abuse was carried out in our laboratory. Random urine samples from a cohort of methadone maintenance patients that had screened positive by immunoassay for the drug of interest were subjected to further confirmatory testing by LC/MS. For the CEDIA Amphetamine/Ecstasy, cross reaction was occurring with other compounds particularly new psychoactive substances such as cathinones.

Objective: To ascertain to what extent positive Amphetamine urine results on CEDIA Amphetamine/Ecstasy Assay were due to cross reactivity with new psychoactive substances.

Methods: The CEDIA Assay kits were set up according to manufacturer's instructions for qualitative screening on Beckman Coulter AU2700 clinical chemistry analysers. The CEDIA Amphetamine/Ecstasy is qualitative with a cut-off level of 1000ng/ml. Reagents, quality controls and calibrators were supplied by Microgenics. Fifty-one urine samples testing positive for Amphetamine class drugs by CEDIA immunoassay were analysed using our in-house stimulant method. This included the following drugs: dimethylcathinone, naphyrone, dimethocaine, 3-fluoromethcathinone, butylone, ethylone, ethcathinone, MDPV, methedrone, 3-TFMPP, BZP, mCPP, 2-aminoindane, 2-phenethylamine, mephedrone, lidocaine, ketamine, caffeine, methylone, MDAI, buphedrone, MDPBP, benzedrone, sildenafil citrate, 4-methylethcathinone, fluorotropacocaine, benzocaine, desoxypipradol, cocaine, benzoylecgonine, methylecgonidine, ethylecgonine, amphetamine, methamphetamine, pseudoephedrine, MDMA, MDA and MDEA. The ABSciex 3200 QTrap method uses 2 MRM transitions for each compound tested and also triggers an EPI giving full scan data. HPLC grade solvents were purchased from Fisher Scientific Ireland. Standards were purchased from LGC where available or provided by Dr Pierce Kavanagh[2].

Results: Of the 51 samples which tested positive by immunoassay for amphetamine, 15 did not contain any of the conventional amphetamine drugs i.e. amphetamine, methamphetamine, MDMA, MDA or MDEA. 11 of the 15 samples contained “Headshop" type drugs (new psychoactive substances). Four showed no presence of amphetamine or the head shop drugs tested; however, as we are only testing for the parent drug there could be metabolites present which would be undetected. Also, given the proliferation of new drugs in the marketplace at the time of this study it is likely that other new psychoactive substances that were not being tested for at the time may have been present in these samples. Drugs detected included 2-aminoindane, mephedrone, methylone , 3-fluoromethcathinone, methedrone, 4-methylethcathinone, dimethocaine, phenethylamine, BZP and 3-TFMPP. Many samples contained multi-drugs with up to four drugs detected in some samples. “Headshop" drugs were also detected in combination with conventional amphetamines.

Discussion: It is known that m-CPP which is a metabolite of Trazodone and also a “Headshop" drug has cross reactivity to Amphetamine immunoassays. We found that the CEDIA Amphetamine/Ecstasy assay may have significant cross reactivity with other compounds, particularly new psychoactive substances such as cathinones. As the method was qualitative and many samples contained multi-drugs, further study is required to determine the level of cross reactivity of individual new psychoactive drugs with the CEDIA Amphetamine/Ecstasy immunoassay.

Conclusion: The CEDIA Amphetamine/Ecstasy assay may have significant cross reactivity with other compounds particularly new psychoactive substances such as cathinones.

Keywords: LCMS, Amphetamine/Ecstasy, Immunoassay, Cross Reactivity, New Psychoactive Substances, Head Shop

P18

Prenatal Tobacco Exposure, Tobacco Biomarkers in Meconium and Infant Outcomes

Sarah K. Himes*[1], Karl B. Scheidweiler[1], Laura Stroud[2], Raymond Niaura[3], and Marilyn A. Huestis[1]; [1]Chemistry and Drug Metabolism, Intramural Research Program, National Institute on Drug Abuse, NIH, Baltimore, MD, USA, [2]Centers for Behavioral and Preventive Medicine, The Miriam Hospital, Department of Psychiatry and Human Behavior, Alpert Medical School, Brown University, Providence, RI, USA, [3]Schroeder Institute for Tobacco Research and Policy Studies, Legacy Foundation, Washington, DC, USA

Introduction: Despite public health warnings, approximately 16% of women smoke tobacco throughout pregnancy. *In utero* nicotine exposure is associated with decreased fetal growth parameters, deficits in cognitive functioning, and increased risk for behavioral disorders and tobacco dependence later in life. Due to these adverse developmental outcomes, identification of exposed infants is important. Meconium testing is often employed to objectively confirm prenatal tobacco exposure.

Objectives: To evaluate the relationship between meconium tobacco marker concentrations and weekly self-reported maternal cigarette consumption and assess the predictive value of tobacco meconium markers on neonatal growth outcomes.

Methods: Women (N=119) enrolled in the Behavior and Mood in Babies and Mothers (BAM BAM) study provided daily tobacco intake levels over pregnancy using timeline follow-back methodology. Cotinine, trans-3'-hydroxycotinine (OHCOT), and nicotine concentrations were quantified in their infants' meconium. LCMSMS limits of quantification (LOQ) were 1.0ng/g cotinine, 5.0ng/g OHCOT, and 2.5ng/g nicotine. Oral fluid specimens collected in the 3rd trimester were analyzed for cotinine by ELISA.

Results: Mothers' median (range) age was 24 years (18-40). The majority (51.1%) of women were Caucasian. Median number of cigarettes smoked daily in the 3rd trimester by smoking mothers was 6.6 (2-20). Meconium specimens from infants born to self-reported non-smoking mothers (N=42) were negative for all tobacco markers, while specimens from self-reported smokers (N=41) were positive for cotinine (80ng/g; 6.4-329), OHCOT (129ng/g; 10.2-478), and nicotine (52.3ng/g; 3.9-294). Quitters (N=28) self-reported stopping smoking in gestational weeks 2-39. Only 4 meconium specimens from infants born to quitters were positive for tobacco biomarkers. These mothers reported quitting during gestational weeks 9, 12, 16 and 39; however, 3rd trimester oral fluid specimens were positive for weeks 9 and 16 suggesting continued smoking. Median meconium concentrations from these 4 infants were cotinine 4.4ng/g (<LOQ-18), OHCOT 20.6ng/g (7.5-30.3) and nicotine 4.3ng/g (<LOQ-37). Infant birth weight, length and head circumference correlated with cotinine, OHCOT, nicotine and total biomarker meconium concentrations among all infants (N=107; Spearman rho -0.32 to -0.39; all p-values<0.041). Among quitters and smokers, reduced infant birth weight and length correlated with total cigarettes smoked and average cigarettes smoked per day in the 3rd trimester (rho -0.25 to -0.34; p-values<0.036).

Conclusions: Results highlight the efficiency of identifying tobacco smoking with high sensitivity meconium LCMSMS analysis for cotinine, OHCOT and nicotine. Reduced infant birth weight, length and head circumference were positively associated with the presence of tobacco biomarkers in meconium and with maternal self-reported cigarette smoking during pregnancy. Data are insufficient to determine whether meconium also reflected 2nd trimester tobacco exposure. Biomarker concentrations and growth parameter associations imply direct toxic exposure effects not likely due to other unmeasured influences. Funded by NIH grant R01 DA019558 and the Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health.

Keywords: Meconium, *In utero*, Tobacco, Smoking, Pregnancy

P19

Method Development and Validation of a Cannabinoid Method that Includes Cannabinol and Cannabidiol Using an Agilent 6430 LC-MS/MS in Positive Ion Mode

Jason S. Hudson*[1], James W. Hutchings[1], Curt E. Harper[1], Patrick N. Friel[2]; [1]Virginia Department of Forensic Science, Richmond, VA, USA, [2]Agilent Technologies, Wilmington, DE, USA

Introduction: Cannabis is one of the most widely used illegal drugs and detected in approximately 27% of DUID cases in the Commonwealth of Virginia. We have developed a LC-MSMS method that quantitates THC, its metabolites, and includes cannabinol and cannabidiol which are potential markers for recent use.

Objectives: To develop and validate a method for the simultaneous detection and quantitation of THC, carboxy-THC, OH-THC, cannabinol, and cannabidiol by LC-MS/MS. We also investigated whether this extraction method measures free or total THC/carboxy-THC.

Methods: Whole blood samples were extracted using 9:1 hexane/ethyl acetate with 10% acetic acid. Extracts were reconstituted in 50:50 acetonitrile/water and the samples separated using an Agilent Technologies 1260 Infinity Series LC with a Poroshell 120 EC-C18, 2.7 µm, 2.1 x 75mm column. The samples were eluted using a gradient of 0.1% formic acid in water and 0.1% formic acid/acetonitrile and analyzed using an Agilent Technologies 6430 triple quadrupole mass spectrometer in positive ion mode. The method development consisted of optimization of chromatography and Masshunter Optimizer® was used to identify the transitions to be used as quantitation and qualifier transitions. A thorough validation of the method was conducted that evaluated linearity; between- and within-day accuracy and precision; interferences; sensitivity; calibration models; carryover; ion suppression; robustness; stability; and previously analyzed case samples.

Results: The calibration range for four of the targets was 0.001-0.100 mg/L and 0.005-0.500 mg/L for carboxy-THC. All targets were best fit by a linear model and the resulting R2 value for the best fit line was >0.991. Accuracy samples were fortified at low, medium, and high concentrations and back calculated concentrations were within 20% of the target concentration with the exception of cannabidiol. The precision studies utilized the accuracy samples in triplicate and the between-run precision CVs were <13% for all targets with the exception of cannabidiol. Within-run precision CVs were <16% for all targets. LOD ranged from 0.001-0.005 mg/L and the LOQ was 0.001mg/L for THC and OH-THC; 0.005mg/L for carboxy-THC. No interferences were detected after analyzing 10 drug classes (46 different drugs) and 13 matrices. Carryover was not detected up to a concentration of 0.5mg/L for all targets except carboxy-THC (2.5 mg/L). Extracted samples were stable over four days and ion suppression and enhancement was determined to be within ±10% for all targets with the exception of cannabinol. All targets met the acceptance criteria with the exception of cannabinol and cannabidiol for our previously analyzed/simulated case results. The glucuronide study revealed that our previous GC-MS method hydrolyzed 35% of carboxy-THC glucuronide adding to the free carboxy-THC concentration.

Conclusions: This method development and validation provides a rapid and sensitive technique for the detection and quantitation cannabinoids. THC, carboxy-THC, and OH-THC met all acceptance criteria. Cannabinol and cannabidiol did not pass the quantitative acceptance criteria but may be evaluated qualitatively. This method measures free carboxy-THC whereas other extraction techniques under basic conditions may measure total carboxy-THC which may have implications in estimation of “time since last use" mathematical models.

Keywords: LC-MS/MS, Cannabinoids, Validation

P20

Development and Validation of a Homogeneous Immunoassay for the Detection of Ethyl Glucuronide in Urine

Guohong Wang*, Kim Huynh, Rehka Barhate, Warren Rodrigues, Christine Moore, Michael Vincent, James Soares; Immunalysis Corporation, Pomona, CA, USA

Introduction and Objective: Ethanol is primarily used as a social drug; however, it can be found in numerous solutions including mouthwash, medical liquids, and manufactured solvent and gasoline additives. Ethanol metabolizes about 95% of its dose and the remaining 5% is unchanged in breath, urine, sweat and feces. Its rapid elimination from the body does not allow for a long detection window following alcohol consumption. Ethyl glucuronide (EtG) is a direct minor metabolite of ethanol and is often used as a biomarker for ethanol intake due to its slow elimination in urine and specificity of ethanol consumption. EtG provides a long window of detection and can be detected up to 130 hours in urine at a cutoff of 500 ng/mL(1). The objective of this project was to develop and validate a high throughput homogeneous enzyme immunoassay (HEIA) for the rapid detection of ethyl glucuronide in human urine.

Methods: An anti-EtG polyclonal based qualitative homogeneous immunoassay was developed and validated for the detection of EtG in urine at two different screening cutoff concentrations: 500 ng/mL and 1000 ng/mL. Some of the assay performance validation included: cross reactivity of structurally related and unrelated compounds; intra-day and inter-day precision; stability; and an analytical performance comparison study using authentic positive and negative specimens which were analyzed using this assay as well as a separate commercially available EtG immunoassay kit and LC-MS/MS.

Results: The intra-day and inter-day coefficients of variation (% CV) for the qualitative assay were less than 1%. The HEIA was validated with a total of 48 urine samples previously analyzed by LC/MS-MS with EtG concentrations range from 250 to 500,000 ng/mL. The sensitivity, specificity and accuracy of the assay were found to be 97%, 94% and 96%, respectively, when an immunoassay cutoff concentration of 500ng/mL was used.

		Confirmation (500 ng/mL)	
		N	P
HEIA (500 ng/mL)	N	16	1
	P	1	30

Conclusion: A high throughput sensitive, specific homogeneous enzyme immunoassay has been developed for the detection of EtG in human urine. When applied to authentic specimens, the assay correlated well with LC-MS/MS results.

References: 1. G. Reisfield; B. Goldberger; B. Crews; A. Pesce; G. Wilson; S. Teitelbaum; and R. Bertholf; Ethyl Glucuronide, Ethyl Sulfate, and Ethanol in Urine after Sustained Exposure to an Ethanol-Based Hand Sanitizer; J. Anal. Toxicol. 35 (2011) 85-91.

Keywords: Ethyl Glucuronide (EtG), Homogeneous Enzyme Immunoassay, Alcohol, Urine Screening, Ethanol Metabolite

P21

Innovative Multi-Drug Single-Run Confirmation Method Using LC/MS/MS Analysis For Drug Testing Laboratories

Thomas David*, James Ritchie, John Roback, Jeff Lavelle, Maya Dobrzeniecka, Scott Damron, Jennifer Blair; Castle Medical, Smyrna, GA, USA

Introduction: A rapid and robust dilute-and-shoot method was developed for screening and quantitating 59 drugs from urine specimens in a single run. Drugs include drugs of abuse, pain medications, antidepressants, stimulants, benzodiazepines and Z-drugs. This was accomplished using simple hydrolysis in conjunction with liquid chromatography tandem mass spectrometry (LC/MS/MS).

Objective: This aim is to develop a lean, highly sensitive and cost effective procedure that allows for the monitoring of multiple drugs within an eight minute run time with minimal sample volume in pain management and drug testing laboratories.

Method: Urine samples were treated with β-Glucuronidase followed by a minimum 90-minute incubation before centrifuging. Calibration standards and controls contain opiates/opioids (27); benzodiazepines (14); amphetamines (4); tricyclic antidepressants (8); illicit drugs (3) and Z drugs (3). Deuterated standards were used as the internal standards in the procedure. High performance liquid chromatography (Schimadzu LC-20) separation utilized gradient elution with a total run time of 8 minutes including a post run equilibration. Using a positive mode of ionization, an ABSciex 3200 triple-quadrupole mass spectrometer was used to monitor the precursor and major product ions for each drug. Sequential MRM mode allowed for monitoring of multiple transitions based on an analyte specific retention time window. The number of data points measured across a chromatographic peak proved to be significantly high enough to achieve good resolution, precision and accuracy. Mobile phase comprised of water and methanol with 0.1% Formic acid. Data analysis was performed on the Analyst software version 1.5.1.

Results: All drugs were analyzed simultaneously using the same procedure steps. Target concentrations varied between 2ng/ml to 20,000 ng/ml among the analytes depending on their therapeutic and toxic levels. Approximately 150 transitions were monitored per run by sequential MRM. Assays with low level cutoffs (such as 20ng/ml for benzodiazepines and 2ng/ml for certain opioids such as fentanyl) were reproducible and met acceptable chromatographic criteria. An accuracy of 95- 105% and a CV of 2.0-10.2 was achieved for each calibration point in a method validation study. Further statistical data indicated an accuracy of 99-108% for quality control samples. The limit of detection and the upper linearity limit results have been quantitated and established for each component.

Discussion: Benefits of the dilute-and-shoot method for urine drug confirmation analysis:

- Rapid analysis process - 8 minute/run
- High number of drugs analyzed concurrently - 59 drugs/run
- Small sample volume required - only 200µl or less/run
- Results show high accuracy and precision for all the analytes
- No extraction or derivatization steps required
- Significantly reduced cost by using less consumables and reagents
- Reduced man hours necessary for sample preparation
- Reduced risk for errors in sample mix-ups and cross contamination

The advantages of this robust, sensitive, and rapid method yield a positive impact on our laboratory's goals in providing an accurate and lean process for the analysis of multiple drugs in a single run.

Keywords: Multiple Drug Confirmation, Dilute-and-Shoot, Lean Rapid Analysis

P22

Analytical Challenges with MS Analysis of Bath Salts and Spice Cannabinoid Metabolites

Joshua Cooper, Ryan Carrell, Ning Chang, Yunming Ying, Greg Kirkovits, **Derrell Johnson***, Isil Dilek, Uma Sreenivasan; Cerilliant Corporation, Round Rock, TX, USA

Introduction: Spice cannabinoids emerged as a regulatory and societal threat in the mid-2000's followed soon after by cathinone-based stimulants. Marketed as “herbal incense" and “bath salts" or “plant food", Spice cannabinoids and synthetic cathinones, respectively, offer recreational highs that mimic illegal drugs such as THC, cocaine, methamphetamine, and LSD. The introduction of new and more potent structural analogs of “legal high" drugs along with their increasing popularity requires the continuous development of new methods for their identification and quantitation by clinical toxicology and forensic laboratories.

Methods: MS remains the tool of choice for method development, detection, and quantitation of new illicit drugs like Spice cannabinoids or bath salt cathinones. While GC/MS and LC/MS provide numerous benefits for these purposes, they also offer challenges unique to the particular technique. GC/MS methods, for example, require use of derivatization reagents for analysis of cathinone-based analogs like bath salts; however, the use of PFPA and BSTFA derivatives with deuterium-labeled internal standards have been reported to cause loss of label in the GC/MS fragmentation. Analysis of matrix-based samples by LC/MS often suffers from interferences or lower ionization efficiency due to matrix effects. While deuterium-labeled internal standards are most commonly used to compensate for matrix effects in LC-MS/MS applications, some labeled compounds may exhibit hydrogen-deuterium scrambling/exchange in the collision cell which necessitates careful selection of MS/MS transitions.

Objective and Results: In this study, we investigated: i) an alternative derivatization method for analysis of the deuterium-labeled bath salts mephedrone, methylone, ethylone, and butylone by GC/MS; and ii) the impact of several variables that potentially contribute to scrambling in the LC/MS analysis of these deuterium-labeled bath salts as well as deuterium-labeled analogs of JWH-073 3-Hydroxybutyl and JWH-018 4-Hydroxypentyl metabolites. The bath salt derivatization method uses TFAA to acetylate the amino group. The free-up procedure is sensitive to choice of base due to instability of the α-amino ketone group found in bath salt cathinones. Derivatization time is critical since decomposition occurs with excessive heating. GC/MS analysis of the TFAA derivatives showed that the deuterium label was retained from the molecular ion to one or two fragmentations. Using the aforementioned deuterium-labeled compounds, we also examined several variables that potentially contribute to scrambling on LC-MS/MS in order to ascertain reproducibility and impact on scrambling ratios - collision energies, deuterium placement in the internal standard, concentration, matrix selection, and influences of different LC/MS systems (tandem quadrupole vs. quadrupole time of flight). Scrambling was observed for the deuterium-labeled Spice metabolites but not for the bath salts. In both cases, scrambling was eliminated by optimizing instrument conditions and transition selection. There is a need, however, to consider potential impact of scrambling on transitions chosen for optimal sensitivity.

Conclusion: Deuterium-labeled internal standards of bath salt cathinones and Spice cannabinoid metabolites are suitable for GC/MS and LC/MS applications when consideration is given to choice of derivatization reagent and transition selection.

Keywords: Bath Salts, Spice Cannabinoids, GC/MS, LC/MS

P23

Development of an Analytical Method for the Investigation of Methylenedioxy Derivatives in Samples of Vitreous Humor in a Traffic Accident

Aline A. Ishikawa[1], **Bruno Spinosa De Martinis*[2]**; [1]University of Sao Paulo/Faculty of Pharmaceutical Sciences of Ribeirao Preto, Brazil, [2]University of Sao Paulo/Faculty of Philosophy, Sciences and Literature of Ribeirao Preto/Department of Chemistry, Brazil

Introduction: The methylenedioxy derivatives 3,4–methylenedioxyamphetamine (MDA), 3,4 methylenedioxymetamphetamine (MDMA, ecstasy) and 3,4-methylenedioxyetilamphetamine (MDEA) are among the illicit use of synthetic drugs most consumed in the occident; substances that cause mental and motor disorders and have caused major threat to traffic safety. These effects affect the performance of drivers and may lead to traffic accidents. The vitreous humor is a biological matrix available in postmortem analysis, and is of great relevance to forensic toxicology. It is a simple matrix, clean easy collection and handling, good stability. It is highly recommended, especially in cases of body injuries, partially charred or in a state of decomposition. This is because the vitreous humor is located in an anatomically isolated area, relatively protected from external contamination and invasion of microorganisms.

Objectives: The purpose of this study was to develop and validate an analytical method to detect and quantify MDA, MDMA and MDEA in samples of vitreous humor in fatal traffic accidents.

Methods: To develop the method we used 1 mL of vitreous humor; liquid-liquid extraction with ethyl acetate solvent; derivatization heptafluorobutyric anhydride (HFBA) and detection by gas chromatography-mass spectrometry (GC/MS).

Results: The method was fully validated and demonstrated linearity from 10 - 400 ng /mL; limits of detection between 1.0 ng/mL and 2.5 ng/mL; accuracy: values between 97.1% - 103.7%; intra-assay precision between 4.54% - 9.14% and inter assay precision between 6.92% -10.59%. The recovery was found greater than 57.87%.

Conclusion: The validated method proved suitable for the investigation of methylenedioxy derivatives in vitreous humor samples using a low cost and simple procedure. The method was applied in the analysis of vitreous humor samples collected from victims of traffic accidents in the region of Ribeirao Preto city.

Keywords: Methylenedioxy Derivatives, Vitreous Humor, Liquid-Liquid Extraction, GC/MS

P24

Analysis of Drugs of Abuse by Laser Diode Thermal Desorption Coupled with Differential Ion Mobility Spectrometry

Adrian Taylor[1]*, Michael Jarvis[1], Hesham Ghobarah[1], Pierre Picard[2], Jean Lacoursiere[2];
[1]AB SCIEX, Concord, Ontario, Canada, [2]Phytronix Technologies Inc., Quebec, Canada

Introduction: (For Research Use Only. Not for Use in Diagnostic Procedures.) A Laser Diode Thermal Desorption ionization source has been coupled to a mass spectrometer equipped with a differential ion mobility cell, enabling a high throughput capacity for the analysis of drugs of abuse in urine, with sample-to-sample analysis time of seven seconds. Sample preparation consisted of a liquid-liquid extraction of urine with ethyl acetate. The lack of separation of isobaric compounds using the LDTD analysis restricted the determination of different opiates of similar mass; however the use of the orthogonal differential mobility spectrometry device allowed separation of these isobarics, and the simultaneous confident identification and quantification of each of the isobaric opiates.

Methods: Liquid-liquid extraction was performed by taking 50 µL of urine and adding 50 µL of NaOH 0.2 N containing of internal standard for each of the drugs. 200 µL Ethyl Acetate was then added and mixed. 2 µL of organic phase was taken and ran on the LDTD-QTRAP® 5500 LC/MS/MS system. The ion source region of the mass spectrometer was modified for incorporation of the differential ion mobility cell. The mass spectrometer analysis consisted of an MRM detection using scheduled MRM algorithm and Enhanced Product Ion dependent scans using the linear ion trap, automatically triggered to collect full scan MS/MS fragmentation spectra. A 1250 compound Forensic Drug Library was searched to provide identification and confirmation.

Results and Discussion: The LDTD uses a Laser Diode to produce and control heat on the sample support which is a 96 well plate. The energy is then transferred through the sample holder to the dry sample which vaporizes prior to being carried by a gas in an APCI region. High efficiency protonation with strong resistance to ionic suppression characterize the ionization due to the absence of solvent and mobile phase. This allows very high throughput capabilities of seven seconds sample-to-sample analysis time without carry over. The SelexION® ion mobility device enables enhanced selectivity over traditional MS/MS analysis by providing an orthogonal means of separating isobaric species using differential mobility spectrometry. This capability becomes especially important when liquid chromatography is not used to separate isobaric species prior to analysis by MS/MS. In the work presented here, the ion mobility cell was interfaced directly to the front of a QTRAP® 5500 mass spectrometer and was optimized for the detection of the drugs of abuse by optimizing the Compensation Voltage (CoV) for each analyte. The isobaric opiate pairs codeine/hydrocodone and morphine/hydromorphone were successfully separated due to their differing compound dependent CoVs to allow determination of the individual analytes without interference from their isobarics.

Keywords: Differential Ion Mobility, Laser Diode Thermal Desorption, LC-MS/MS

P25
Evidence of Cathinones Use Through Hair Analysis Using LC-MS/MS

Aymeric Morla[1], Mathieu Martin[2], Kirsten Turner[2], Mathieu Duez[2], Nadine Klinger[2], Vincent Cirimele[2], **Adrian Taylor*[3]**; [1]AB SCIEX, Les Ulis, France; [2]Laboratoire ChemTox, Illkirch, France; [3]AB SCIEX, Concord, Canada

Introduction: (For Research Use Only. Not for use in diagnostic procedures.) During the last few years, there has been a rapid growth in the availability of new synthetic substances (commonly known as 'legal highs') in the UK and throughout Europe, the majority of which are freely available over the internet or in 'head shops'. Mephedrone and related cathinone derivatives were banned in the UK in April 2010. We have developed and validated a novel method for a range of cathinones in hair by LC-MS/MS to include cathinone; methcathinone; methylethcathinone; mephedrone (4-methylmethcathinone); methedrone/bk-PMMA (methoxymethcathinone); MDPV (methylenedioxypyrovalerone); butylone/bk-MBDB (methylamino-1-(3;4-methylenedioxyphenyl) butan-1-one) and methylone/bk-MDMA (2-Methylamino-1-(3;4-methylenedioxyphenyl) propan-1-one.

Methods: After decontamination of the hair strand in methylene chloride, segments were cut into small pieces with scissors, weighed and incubated in methanol overnight at 4°C in the presence of internal standard. After sonication and centrifugation, the supernatant was recovered and evaporated to dryness. After reconstitution in 1 mL of acetonitrile/0.1% formic acid in 2mM ammonium formate buffer (5:95), 10 µL were injected. Chromatographic separation was achieved on a Kinetex XB-C18 (2.1 x 100mm; 2.6µm) column. The LC-MS/MS system consisted of a Shimadzu UFLC-XR coupled to an AB SCIEX QTRAP 5500® using Positive Electrospray Ionisation. The acquisition was done in Scheduled MRM mode. The method was validated according to the parameters stated within standard operating procedures for method validation (ISO 17025).

Results and Discussion: Linearity was verified from 50 pg/mg to 20 ng/mg. Inter and intra-day variability (%CV) were less than 10% at 0.5 and 2 ng/mg. Sixty-one hair specimens were screened for cathinones. Twenty-one were found positive for mephedrone with concentrations ranging from 0.1 to 36 ng/mg with a median concentration of 0.42 ng/mg. The sample reading 36 ng/mg was re-analysed on a lower volume of hair sample as it was above the highest linearity point. These concentrations are in accordance with results previously produced by this laboratory for mephedrone by GCMS. MDPV and methedrone were also detected in two cases at trace levels. The cathinones seem to be well incorporated in hair and the method is sensitive enough to be able to detect cathinones in subjects where occasional or regular use is suspected. The mephedrone concentrations are in the ng/mg range like other psychoactive drugs such as amphetamines. The method performs well when screening (direct solvent incubation) a large number of samples.

Keywords: Hair Analysis, LC-MS/MS, Cathinones

P26

Automation of a Method for the Analysis of a Pain Panel by LC-MS/MS: From Sample to Report

Adrian Taylor*[1], Michael Jarvis*[1], Shirk Mohammed[2], Markus Wiggli[3]; [1]AB SCIEX, Concord, Canada, [2]Tecan, Research Triangle Park, NC, USA, [3]Tecan, Mannedorf, Switzerland

Introduction: (For Research Use Only. Not for Use in Diagnostic Procedures.) Liquid chromatography-tandem mass spectrometry technology provides laboratories with a powerful tool for robust, accurate, sensitive detection of a wide variety of analytes. However, in the absence of complete method automation, results are susceptible to human error at many different stages including preparation of calibration standards, sample preparation, and data processing.

Objective: The objective of this work was the automation of an LC-MS/MS method for the analysis of a pain panel comprising 15 analytes, to eliminate human error, increase reproducibility, eliminate subjectivity during data processing, and save time.

Methods: In the work presented here, an LC-MS/MS method for the analysis of a pain panel comprising 15 analytes has been developed and automated from start to finish. Calibration stock solutions and individual calibration standards were automatically prepared using a Tecan Freedom Evo 150 liquid handling system, and pre-analytical sample preparation was performed on the liquid handling system. Urine samples were loaded onto the Tecan system in 13mm test tube racks, and the final samples were prepared in a 96-well plate format, which was transferred to the LC-MS/MS system for analysis. The LC-MS/MS system consisted of a Shimadzu Prominence HPLC system interfaced to an AB SCIEX API 3200™ triple quadrupole mass spectrometer. The data acquisition, processing, and reporting was performed using the Cliquid® software, which enabled the direct import of batch lists from the Freedom Evo liquid handling system. Upon completion of data acquisition, the software automatically performed quantitation for the 15 analytes included in the method, and automatically generated and printed out reports.

Results and Conclusion: The reproducibility of the automated protocol was assessed by preparing and analyzing replicates of each calibration standard, and the measured CVs were less than 15% over the entire concentration range covered by the assay. The method displayed good linearity for all analytes, with R>0.999.

Keywords: LC-MS/MS, Automation, Sample Preparation

P27
High Sensitivity Analysis of THC-COOH and THC in Oral Fluid

Brent Dawson*[1], Witold Woroniecki[1], Xiaohong Chen[1], Adrian Taylor[2]; [1]AB SCIEX, Foster City, CA, USA, [2]AB SCIEX, Concord, Canada

Introduction: (For Research Use Only. Not for use in Diagnostic Procedures.)
Oral fluid is becoming increasingly popular as a sample for forensic drug testing. While the levels of THC in oral fluid pose no great challenge for LC-MS-MS analysis, the analysis of metabolites is often preferred to rule out passive exposure. Unfortunately, THC-COOH is present at very low levels (<100 pg/mL), and oral fluid collection devices limit the amount collected and dilute the sample in a stabilization solution containing surfactants. In this work, we demonstrate a method that utilizes a sensitive and fast hybrid triple quadrupole linear ion trap mass spectrometer to determine THC-COOH and THC in oral fluid. The method gives a LLOQ of 10 pg/mL for THC-COOH and 200 pg/mL for THC in oral fluid.

Methods: Aliquots of 245 µL of human oral fluid were spiked with 5µL THC-COOH and THC stock in methanol to a calibration curve from 8-5000 pg/mL of THC-COOH and 200-25,000 pg/mL of THC. A 750 µL volume of extraction buffer (Immunalysis) was added to the standards, and all samples, standards and controls were acidified prior to analysis. Solid phase extraction was performed on all standards and controls prior to injection. The overall runtime was 6.1 minutes including all column wash and equilibration steps. The three MRM transitions were monitored per analyte (THC-COOH: 344.2>299.2; 344.2>245.1; 344>191.1 THC:315.2>297.2; 315.2>193.1; 315.2>123.1 and ratios were performed to ensure proper peak identification.

Results and Discussion: While THC-COOH can be analyzed with positive ionization at levels >500 pg/mL, negative ionization was needed for high sensitivity analysis. Since THC ionizes better in positive mode, the polarity switching feature of the hybrid triple quadrupole linear ion trap was utilized. Excellent linearity down to 10 pg/mL was readily achieved in neat solvent; but initial studies showed poor linearity in the oral fluid/extraction buffer matrix. Multiple strategies were taken to resolve isobaric interferences from the analyte peaks including solid phase extraction and the use of more selective mrm transitions. In the end; both approaches were needed. The method gives a LLOQ of 10 pg/mL for THC-COOH and 200 pg/mL for THC in oral fluid and is linear up to 5 ng/mL for THC-COOH and 25 ng/mL THC. It requires no derivatization and can be readily automated for high through-put analysis.

Keywords: Forensics, Oral Fluid, THC

P28

Identification of Specific Cytochrome P450 Isozymes Mediating Biotransformation of Three Common Drugs of Abuse

Kevin J. Schneider* and Anthony P. DeCaprio; Department of Chemistry and Biochemistry and International Forensic Research Institute, Florida International University, Miami, FL, USA

Background: Knowledge of the specific enzymology behind drug biotransformation is of great importance in medicine and forensics, especially in examining drug-drug interactions, toxicity mediation, reactive metabolite generation, stimulation of addiction pathways, and pharmacogenomics. While complete investigation into these pharmacological properties is commonly performed for manufactured pharmaceuticals, similar data for illicit abused substances are not generally available.

Objective: The present research evaluates primary Phase I biotransformation pathways for cocaine, methamphetamine, and morphine, with determination of the cytochrome P450 (CYP) isoforms responsible for each specific reaction.

Methods: Cocaine, methamphetamine, and morphine were subjected to metabolic profiling using panels of in vitro assays containing heterologously expressed human CYP bactosomes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4). Sample mixtures contained a specific CYP bactosome, drug substrate (cocaine, methamphetamine, or morphine), cofactors ($MgCl_2$ and NADPH), and a NADPH regeneration system in pH 7.4 sodium phosphate buffer. After incubation for 30 min at 37°C, samples were quenched and proteins denatured by addition of acetonitrile, and supernatants were recovered after centrifugation. Following vacuum evaporation of supernatants, metabolites were reconstituted in solvent containing internal standard (benzoylecgonine-D_3, methamphetamine-D_5, or morphine-D_3), and were subsequently analyzed by LC-MS/MS on an Agilent 1290/6460 LC-QQQ-MS instrument using multiple reaction monitoring.

Results: MS/MS analysis confirmed the influence of several CYP isozymes already cited in the literature and revealed additional enzymatic contributions not previously reported for each of the three drugs examined. In accordance with previous reports [1], cocaine metabolism by CYP3A4 was confirmed as a major pathway. In addition, CYP2C8 and CYP2C19 were found to mediate cocaine biotransformation. As previously reported [2], methamphetamine biotransformation was found to be mediated by CYP2D6, but new data also indicate involvement of CYP2C19. Novel findings also include evidence for regioselective activity of specific P450 isozymes in the aromatic hydroxylation of cocaine and methamphetamine. In addition, Phase I metabolism of morphine was found to be mediated primarily by CYP2C8 and CYP2C19.

Conclusion: Results of this research are in general agreement with earlier reports [1,2] and also provide new data on additional specific CYP isoforms involved in metabolism of these important drugs of abuse. In addition, the data confirm the value of in vitro models for comprehensive elucidation of Phase I metabolic profiles for such drugs. Finally, the findings regarding cocaine metabolism by CYP2C19 may provide support for a link between increased CYP2C19 expression and previously suggested [3] physiological pathways associated with cocaine addiction.

References:

[1] LeDuc, B.W.; Sinclair, P.R.; Shuster, L.; Sinclair, J.F.; Evans, J.E.; Greenblatt, D.J. Pharmacology, 46 (1993) 294-300.
[2] Lin, L.Y.; Di Stefano, E.W.; Schmitz, D.A.; Hsu, L.; Ellis, S.W.; Lennard, M.S.; Tucker, G.T.; Cho, A.K. Drug Metab. Dispos., 25 (1997) 1059-1064.
[3] El-Moselhy, T.F.; Avor, K.S.; Basmadjian, G.P. Med. Chem. Res., 10 (2000) 50-57.

Keywords: Cocaine, Methamphetamine, Morphine, Cytochrome P450 Isozymes, Pharmacogenomics, In Vitro Assay

P29

The Analysis of Common Drug of Abuse in an Oral Fluid Matrix Using A New QTRAP® Platform

Jesse C. Seegmiller[1], Xiaohong Chen[1], **Adrian Taylor*[2],** Hua-fen Liu[1]; [1]AB SCIEX, Foster City, CA, USA, [2]AB SCIEX, Concord, Canada

Introduction: (For Research Use Only. Not for Use in Diagnostic Procedures.) Much interest has surrounded the detection of several pain medications in oral fluid matrices. What makes oral fluid an attractive matrix is the fact that it involves a non-invasive collection. The collection of such a matrix typically involves a collection swab that retains the oral fluid matrix. Once collection has been completed, the drugs are then removed from the swab using an extraction and preserve buffer. Using LC/MS/MS, we have developed a simple dilute and shoot method to simultaneously quantify these compounds in an oral fluid matrix. The compounds analyzed in this method consist of nine drugs including: morphine, 6-MAM, Amphetamine, Benzoylecgonine, Codeine, MDA, MDMA, Methamphetamine and PCP.

Methods: The above nine compounds were spiked into an oral fluid matrix at various levels. The oral fluid matrix collection workflow was simulated by diluting with an extraction buffer at the appropriate dilution factor. This solution was then further diluted with a methanol:water diluent prior to drug analysis. The above extract was then analyzed on reversed phase chromatography employed using the MPX™-2 high throughput multiplexed HPLC system and compounds were detected with a QTRAP® new platform operating in positive electrospray ionization mode. Use of the MPX™-2 system afforded less than 5 minutes per sample and potentially double the throughput of a single LC- setting.

Results and Discussion: Two MRM transitions were used for each compound; one for quantitation and one for qualification. Calibration curve was constructed to include the nine drug panel and was found to be linear in the following range in the oral fluid matrix: 6-MAM 0.75 - 10 ng/mL; Amphetamine 7.81-500 ng/mL; Benzoylecgonine 2.34-150 ng/mL; Codeine 3.125-100 ng/mL; Morphine 6.25-100 ng/mL; MDA 3.91-250 ng/mL; MDMA 3.91-250 ng/mL; Methamphetamine 7.81-500 ng/mL and PCP 0.39-25 ng/mL.
Calibrators prepared in solvent solutions were used to determine absolute recovery from oral fluid matrix with this method. Recovery for all compounds was found to be within 80-100%. The stability of the compounds in the matrix was also evaluated and will be discussed.

Keywords: Oral Fluid, LC-MS/MS, Drugs of Abuse

P30

A Simple LC/MS/MS Screening and Quantify Method for the Analysis of 41 Common Pain Drugs in an Oral Fluid Matrix

Xiaohong Chen*[1], Jesse C. Seegmiller[1], Jenny E. Moshin[1], Michael Shaw[1], Adrian Taylor[2], Hua-fen Liu[1]; [1]AB SCIEX, Foster City, CA, USA, [2]AB SCIEX, Concord, Canada

Introduction: (For Research Use Only. Not for Use in Diagnostic Procedures.) Currently there is much interest associated with the analysis of various pain medications in oral fluid matrices. Typical collection of such a matrix involves a collection swab that retains the oral fluid matrix. The swab is then subjected to extraction buffer before sending to laboratories for analysis. Therefore, we have developed a simple method to simultaneously screen and quantify such compounds in one injection using a new QTRAP® platform. The compounds analyzed in this method consists of 41 drugs: 6-MAM; Alprazolam; Amphetamine; Benzoylecgonine; Buprenorphine; Carisoprodol; Clonazepam; Codeine; Diazepam; EDDP; Fentanyl; Flunitrazepam; Flurazepam; Hydrocodone; Hydromorphone; Hydroxyalprazolam; Lorazepam; MDA; MDEA; MDMA; Meperidine; Meprobamate; Methadone; Methamphetamine; Midazolam; Morphine; Naltrexone; Norbuprenorphine; Nordiazepam; Norfentanyl; Normeperidine; Norpropoxyphene; Oxazepam; Oxycodone; Oxymorphone; PCP; Propoxyphene; Sufentanil; Temazepam and Tramadol.

Methods: An oral fluid matrix was spiked to various levels of the above drugs. The calibration curve extended above and below the cutoff and confirmation levels. Sample preparation procedure involves diluting oral fluid matrix with extraction buffer at the appropriate dilution factor. An aliquot of the above solution was then analyzed using a new QTRAP® platform operating in MRM coupled with enhanced product ion scan in one injection for library searching as screening. The liquid chromatography system used was the MPX-2 high throughput multiplexed HPLC system to achieve maximum throughput. By using the MPX-2 system analysis, we were able to achieve analysis times of less than 5 minutes per sample.

Results and Discussion: Exceptional upper and lower limits of quantitation (ULOQ and LLOQ) were achieved while maintaining chromatographic separation for common isobaric interfering compounds; e.g. Codeine and Hydrocodone; Morphine and Hydromorphone. Two MRM transitions were used for each compound; one for quantifier and one for qualifier. Calibration curve was constructed to include the 41-drug panel and was found to be linear in the following range in the oral fluid matrix. The enhanced product ion spectrum was used for library searching as screening results. The report was automatically generated by a reporter and the results can be directly uploaded to different LIMS system. The following linear ranges were obtained from the oral fluid analysis: 6-MAM 0.75-10 ng/mL; Alprazolam 1.56-100 ng/mL; Amphetamine 7.81-500 ng/mL; Benzoylecgonine 2.34-150 ng/mL; Buprenorphine 12.5-100 ng/mL; Carisoprodol 3.125-200 ng/mL Clonazepam 3.125-100 ng/mL; Codeine 3.125-100 ng/mL; Diazepam 3.125-100 ng/mL; EDDP 1.56-100 ng/mL; Fentanyl 0.094-3 ng/mL; Flunitrazepam 3.125-100 ng/mL; Flurazepam 3.125-100 ng/mL; Hydrocodone 3.125-100 ng/mL; Hydromorphone 3.125-100 ng/mL; Hydroxyalprazolam 3.125-100 ng/mL; Lorazepam 3.125-100; MDA 3.91-250 ng/mL; MDEA 3.91-250 ng/mL; MDMA 3.91-250 ng/mL; Meperidine 15.625-1000 ng/mL; Meprobamate 6.25-200 ng/mL; Methadone 3.125-100 ng/mL; Methamphetamine 7.81-500 ng/mL; Midazolam 3.125-100 ng/mL; Morphine 6.25-100 ng/mL; Naloxone 6.25-100 ng/mL; Naltrexone 6.26-100 ng/mL; Norbuprenorphine 3.125-100 ng/mL; Nordiazepam 3.125-100 ng/mL; Norfentanyl 0.3125-10 ng/mL; Normeperidine 1.5625-100 ng/mL; Norpropoxyphene 4.69-150 ng/mL; Oxazepam 3.125-100 ng/mL; Oxycodone 3.125-100 ng/mL; Oxymorphone 6.25-100 ng/mL; PCP 0.39-25 ng/mL; Propoxyphene 2.34-150 ng/mL; Sufentanil 0.094-3 ng/mL; Temazepam 3.125-100 ng/mL; Tramadol 1.56-100 ng/mL. Calibrators prepared in solvent solutions were used to determine absolute recovery from oral fluid matrix with this method. Recovery for all compounds was found to be within 80-100%.

Keywords: Pain Drugs, Oral Fluid, Multiplexing

P31

Monitoring Drug Abuse of Patients in Substitution Therapy: Comparison of UPLC-MS/MS Screening in Oral Fluid and Urine Testing with Immunoassay

Michael Boettcher*, Stefan Lierheimer; MVZ fuer Mikrobiologie, Labordiagnostik und Hygiene Dessau GmbH, Dessau, Germany

Introduction: Screening for drugs of abuse with immunoassay in urine samples from patients in heroin substitution treatment can be regarded as standard practice. Oral fluid (OF) gains increasing interest in drugs of abuse testing of these patients because of ease of collection and less risk for adulteration. However, little is known about the required sensitivity of the possible screening methods to reach comparable positive rates or so to speak similar detection times. We therefore developed a sensitive UPLC-MS/MS method for OF screening and compared the positive rates to standard urine testing of substituted outpatients from the Berlin area.

Methods: Of samples were collected using the Greiner-Bio-One SCS pH 4.2 device. OF concentration of the OF/buffer mixture was quantified spectrophotometrically on an Olympus AU680. Urine screening was conducted on an Olympus AU680 with immunoassays from Thermo Fisher at following cutoffs: amphetamines 500 ng/mL, benzodiazepines (with enzymatic hydrolysis) 100 ng/mL, cocaine 50 ng/mL, opiates 100 ng/mL, EDDP 100 ng/mL, buprenorphine 2 ng/mL, THC-COOH 25 ng/mL. SRM multi-target drug screening in OF was performed after LLE of 0.5 mL sample and separation on an UPLC/MS-MS (Waters Acquity and Xevo TQ) with a BEH Phenyl 1.7 µm, 2.1 mm x 100 mm column within 6 minutes. OF/buffer was spiked to 0.5 ng/mL with the corresponding deuterated standards. Two transitions were monitored for the following 47 analytes: amphetamines (10), benzodiazepines (16), cocaine, benzoylecgonine, opiates including 6-acetylmorphine (6), opioids (5), THC, methadone, buprenorphine, others (5). The cutoff in neat saliva was set at 0.1 ng/mL for buprenorphine and at 1 ng/mL for all other analytes.

Patients: During a three month period OF and urine drug testing positive rates were compared from 1. An outpatient clinic (OPC) where the drug testing was stepwise moved from urine to OF and from 2. Other outpatient clinics (ALL) with more random selection between the two matrices. Patient data: OPC: 194 patients (26 buprenorphine, 67 methadone, 101 polamidone), 902 OF samples and 182 patients (25 buprenorphine, 66 methadone, 91 polamidone), 1119 urine samples. ALL: 612 patients from 23 clinics (116 buprenorphine, 265 methadone, 231 polamidone), 1072 OF samples and 1463 patients from 40 clinics (285 buprenorphine, 673 methadone, 505 polamidone), 9008 urine samples.

Results: The positive rates for OF/urine samples from OPC were: amphetamines: 9.3%/3.3%, benzodiazepines: 11.0%/14.4%, cocaine: 5.2%/3.9%, opiates: 13.5% (76% positive for 6-acetylmorphine)/13.5%, opioids + others: 2.0%/not tested and THC: 26.9%/THC-COOH: not tested. Methadone/EDDP was positive in both matrices where expected. However, buprenorphine was negative in 8 OF samples from 2 patients in low dose therapy (0.4 and 1.0 mg/d). The positive rates for OF/urine samples from ALL were: amphetamines: 10.3%/4.1%, benzodiazepines: 25.7%/22.4%, cocaine: 9.8%/7.2%, opiates: 17.6% (76% positive for 6-acetylmorphine)/21.7%, opioids + others: 3.5%/not tested and THC: 30.5%/THC-COOH: 31.3%. Methadone/EDDP and buprenorphine was positive in both matrices where expected.

Conclusions: The positive rates for OF and urine were comparable at the selected cutoffs suggesting that OF is of equal value. The 0.1 ng/mL cutoff for buprenorphine in OF needs further evaluation for patients in low-dose therapy.

Keywords: Oral Fluid, Multi-Target Drug Screening, Positive Rates

P32
Abstract Withdrawn

P33

Development of a Highly Sensitive Generic Polyclonal Antibody for the Detection of Phenylacetylindole Synthetic Cannabinoids: JWH-250, JWH-251, JWH-203 and JWH-249

K. McConville, J. Taggart, **M.E.O. Benchikh***, R.I. McConnell, S.P. Fitzgerald; Randox Toxicology Limited, Crumlin, UK

Background: JWH-250 [1-pentyl-3-(2'-methoxyphenylacetyl) indole] and related compounds, JWH-251 (2'-methyl), JWH-203 (2'chloro) and JWH-249 (2'-bromo) are analgesic chemicals from the phenylacetylindole family. They act as cannabinoid agonists at both the CB1 and CB2 receptors. Unlike many of the older JWH series compounds, these compounds do not have a naphthalene ring; instead, occupying this position are phenylacetyl groups (2'-methoxy; 2'-methyl; 2'-chloro; 2'-bromo), making them representative members of a new class of cannabinoid ligands. JWH-250 and family were discovered by and named after Dr. John W. Huffman. They are research chemicals and, as such, are not approved for human consumption as currently insufficient data exists about their side effects; adverse reactions; long term damage and potential for addiction.

Objective: The aim of this study was to develop the first highly sensitivity and generic polyclonal antibody against the phenylacetylindole family of synthetic cannabinoids - JWH-250; JWH-251; JWH-203 and JWH-249. This antibody could be used for the development of a generic immunoassay for the determination of these compounds in human samples.

Methods: The immunogen comprising JWH-250 hapten coupled to bovine thyroglobulin (BTG) as carrier protein was administered to adult sheep on a monthly basis to provide target-specific polyclonal antiserum. IgG was extracted from the antiserum and evaluated via competitive immunoassay.

Results: The polyclonal antibody generated to the JWH-250-BTG immunogen showed a half maximal inhibitory concentration (IC50) of 2.7ng/ml, with a calibration range of 0-120ng/ml for JWH-250 (100% cross reactivity). It also presented cross- reactivity with other members of the phenylacetylindole family: JWH-251 (123.7% cross reactivity), JWH-203 (68% cross reactivity) and JWH-398 (21% cross reactivity).

Conclusion: The polyclonal antibody developed is highly sensitive and presents broad specificity profile against the phenylacetylindole family of synthetic cannabinoids. This antibody is suitable for the development of generic immunoassays for the determination of these compounds in human samples; with important forensic and toxicological applications.

Keywords: Synthetic Cannabinoids, JWH-250, Generic Antibody, Immunoassay

P34

Determination of Amphetamines, Cathinones and Piperazines in Urine by Mixed Mode Solid Phase Extraction and LC-MS/MS

Rui Shen Ong, Maria Devianna Avisena Lieka, **Hooi Yan Moy***, Yi Ju Yao, Chi Pang Lui; Drug Abuse Testing Unit, Analytical Toxicology Laboratory, Analytical Toxicology Division, Applied Sciences Group, Health Sciences Authority, Singapore

Background/Introduction/Objective: Designer drugs belonging to the classes of beta-keto-amphetamines and piperazines have emerged as attractive alternatives for illicit amphetamines-type recreational drugs worldwide due to their similarity in psychotropic effects. In Singapore, 4-methylmethcathinone (4MMC), 1-benzylpiperazine (BZP) and 1-(3-trifluoromethylphenyl)piperazine (TFMPP) have been classified as Class A Controlled Drugs in the First Schedule of the Misuse of Drugs Act (CAP. 185) since November 2010. As these compounds are used either as substitutes for or in conjunction with amphetamines, it is necessary for our laboratory to develop a high-throughput screening method aimed at detecting an extensive range of controlled substances in a single run. The goal of the study is to develop a LC-MS/MS method for the identification and quantification of Cathinone (CAT), Methcathinone (MC), 4MMC, Methylone, Butylone, BZP, TFMPP, Amphetamine (AMP), Methamphetamine (METH), α-methyl-3,4-(methylenedioxy)phenethylamine (MDA), N,α-dimethyl-3,4-(methylenedioxy)phenethylamine (MDMA) and N-ethyl-α-methyl-3,4-(methylenedioxy)phenethylamine (MDEA) in urine.

Materials and Methods: Extraction of urine samples was performed on mixed-mode EVOLUTE® CX solid phase extraction columns in the 96-well format.

Sample Pretreatment: 500 µL of urine + 50 µL of internal standard solution containing 500 ng/mL each of D_5-AMP, D_8-METH, D_5-MDA, D_5-MDMA, D_6-MDEA, D_7-BZP, D_4-TFMPP and D_3-4MMC + 1 mL NH_4OAc buffer (0.05 M; pH~6)
Column Conditioning: 1 mL MeOH followed by 1 mL NH_4OAc buffer (0.05 M; pH~6)
Interference Wash 1: 1 mL 2% formic acid in water; Interference Wash 2: 1 mL MeOH
Elution: 1 mL 5% NH_4OH in MeOH

100 µL methanolic HCl was added to each well of the collection plate prior to elution to minimize analyte losses during the drying step. The extracts were evaporated to dryness and reconstituted in 100 µL 98:2 (v/v) 10 mM ammonium formate (mobile phase A): acetonitrile (mobile phase B) for LC-MS/MS analysis. A Waters UPLC-MS/MS system comprising of an Acquity ultra performance liquid chromatograph and Quattro Premier Tandem Mass Spectrometer was used. The chromatographic separation was achieved on a reversed phase column (Aquity BEH C_{18} column 100 x 2.1 mm id; particle size 1.7 µm) in a 10 min runtime using gradient elution of mobile phases A and B with an injection volume of 2 µL. Data acquisition was performed in multiple reaction monitoring (MRM) mode with positive electrospray ionization (ESI+). One MRM transition was monitored for the internal standards whereas the targeted analytes were detected using either two or three MRM transitions.

Results and Conclusions: The assay was found to be linear from 1 to 500 ng/mL for all analytes studied. The LODs (S/N > 3) and LOQs (S/N > 10) ranged from 0.1 to 5 ng/mL and from 10 to 50 ng/mL respectively. Matrix effects and extraction efficiencies were found to range from 63.9% to 94.0% and 98.6% to 110.7% respectively. Within-day and between day imprecision were within acceptance limits of ≤ 20%. Accuracy data were also within the acceptance interval of ± 20% of expected concentrations. The selectivity of the method was assessed for the potential interferences by other common drugs of abuse or related amphetamines-type drugs typically taken in combination. This validated LC-MS/MS method was found to be appropriate for the simultaneous quantification of the analytes of interest in urine at low levels.

Keywords: Amphetamines, Cathinones, Piperazines, LC-MS/MS, Urine Drug Screening

P35

Development of a Biochip Array for the High Sensitivity Multiplex Screening of Chloral Hydrate Metabolite, Ethyl Glucuronide, Fentanyl, Flunitrazepam, Ketamine Metabolite, Meperidine, Meprobamate and Z Drugs in Whole Blood

J. Darragh*, R. Acheson, E. McShane, D. McAleer, R.I. McConnell, S.P. Fitzgerald; Randox Toxicology Limited, Crumlin, UK

Background: Some prescription sedatives and alcohol are commonly associated with overdose and sexual assault. In test settings, the availability of screening methods allowing simultaneous detection of these compounds is advantageous as more test results are obtained from a sample and only positive test results need to be assessed by a confirmatory method. Evidence biochip array technology provides a platform for the simultaneous determination of multiple analytes from a single sample.

Objective: This study reports the development of a high sensitivity biochip array for the simultaneous screening of chloral hydrate metabolite; ethyl glucuronide; fentanyl; ketamine metabolite; flunitrazepam; meperidine; meprobamate; zaleplon; zolpidem and zopiclone in whole blood.

Methods: Ten simultaneous competitive chemiluminescent immunoassays; defining discrete test sites on the biochip surface were applied to the Evidence Investigator analyser. The biochip is also the vessel for the immunoreactions. The system incorporates the dedicated software to process, report and archive the data generated.

Results: The specificity profiles of the immunoassays on the biochip were as follows: the chloral hydrate metabolite assay was standardized to urochloralic acid (trichloroethyl beta D glucuronide) and was specific to urochloralic acid (trichloroethyl beta D glucuronide). The ethyl glucuronide assay was standardized to ethyl glucuronide and presented %cross-reactivity of 5% (methyl glucuronide); 9% (methylethylglucuronide); no cross-reactivity with trichloethyl B-D glucuronide was observed. The fentanyl assay was standardized to fentanyl and presented %cross-reactivity 59.5% with benzylfentanyl. The flunitrazepam assay was standardized to 7 amino flunitrazepam and the cross-reactivity values for flunitrazepam and diazepam were 79% and 6% respectively. The ketamine metabolite assay was standardized to norketamine; the %cross-reactivity value with ketamine was 2%. The meperidine assay was standardized to normeperidine; the %cross-reactivity with meperidine was 182%. The meprobamate assay was standardized to meprobamate; the %cross-reactivity with carispoprodol was 88%. The Zaleplon assay was specific for the target analyte. The zolpidem assay was standardized to zolpidem and presented a %cross-reactivity value of 31% with phenyl-4-carboxyzolpidem. The zopiclone assay was standardized to zopiclone and presented %cross-reactivity values of 141% (N-desmethyl zopiclone); 135% (zopiclone N-oxide) and 22% (eszopiclone). The limit of detection values (LOD) in whole blood were as follows: 92ng/ml for chloral hydrate metabolite; 88ng/ml for ethyl glucuronide; 0.2ng/ml for fentanyl; 0.4ng/ml for flunitrazepam; 1.9ng/ml for ketamine metabolite; 0.2ng/ml for meperidine; 10ng/ml for meprobamate; 0.4ng/ml for zaleplon; 0.3ng/ml for zolpidem and 1.5ng/ml for zopiclone in whole blood. The assays exhibited intra-assay precision and inter-assay precision, expressed as %CV, ranging from 4.5 to 13.2% and from 6.1 to 16.3% respectively.

Conclusion: The data indicate applicability of the developed biochip array to the simultaneous screening of chloral hydrate metabolite; ethyl glucuronide; fentanyl; ketamine metabolite; flunitrazepam; meperidine; meprobamate; zaleplon; zolpidem and zopiclone in whole blood. This screening tool facilitates the test process as the number of test results from a sample is increased and only positive test results need to be confirmed,which is of interest in forensic toxicology and drug facilitated sexual assault cases.

Keywords: Evidence Biochip Array Technology, Simultaneous Screening, Chemiluminescent Immunoassays

P36

High Throughput Confirmation Method of Amphetamine and Methamphetamine in Urine by Laser Diode Thermal Desorption (LDTD) Combined to a Mass Spectra System (MS/MS)

Serge Auger*, Pierre Picard, Alex Birsan, Gregory Blachon; Phytronix Technologies Inc., Quebec, QC, Canada

Objectives: Amphetamine and Methamphetamine are psycho stimulant drugs of the phenethylamine class that produces increased wakefulness and focus in association with decreased fatigue and appetite. Detection and quantification of Amphetamine and Methamphetamine in urine is traditionally performed by LC/MS/MS or GC/MS analysis. The Laser Diode Thermal Desorption (LDTD) system combined with a Mass Spectra (MS/MS) allows analysis in ten seconds sample to samples. We propose to validate a confirmation method for Amphetamine and Methamphetamine in urine at a concentration ranging from 50 to 2500 ng/mL.

Materials and Methods: A calibration curve, quality control material, and patient specimens are spiked with Internal Standard containing Amphetamine-D5 and Metamphetamine-D9. A Liquid-Liquid extraction (LLE) is performed in basic condition using ethyl acetate: 100 µL urine; 20 µL internal standard solution in Methanol; 100 µL NaOH (0.1N in water) and 600 µL Ethyl Acetate. 100 µL of organic phase is transferred and mixed with 25µL HCl (0.4N in methanol). A volume of 5µL of this mixture is deposited in 96-well plate made for LDTD-MS/MS analysis.

Results: Use of HCl into solution before spotting 96-well plate decreases the compound evaporation effect at room temperature (volatile amine molecule) allowing accurate analysis of dry compound. The calibration curves show excellent linearity with r^2 of 0.9994 and 0.9975 for Amphetamine and Methamphetamine, respectively. The limits of quantification were established to be 50 ng/mL for both analytes, while the upper limit of linearity was set at 2500 ng/ml for both compounds. This method is cross validated with the gold standard, LC-MS/MS, over 23 patient specimens. All samples within the quantification range correlated within the acceptance criteria of ±20 % and the correlation between LC-MS/MS and LDTD-MS/MS analysis gives excellent $r^2 > 0.99$. All negative samples correlated accordingly. The intra-run and inter-run imprecision was evaluated to be less than 6.3 % for both analytes. The wet stability was evaluated to within 4-days and the Lazwell plate (dry sample) stability to be 3-days.

Conclusions: LDTD technology provides unique advantages in developing an ultra fast method for analysis of Amphetamine and Methamphetamine in urine. This method has demonstrated accurate, precise and stable results at a speed 30-time faster (10 second vs 5 minutes) than the LC-MS/MS method.

Keywords: High Throughput, LDTD-MS/MS, Amphetamine, Methamphetamine

P37
Abstract Withdrawn

P38

Fast Screening of Drugs in Urine by Laser Diode Thermal Desorption (LDTD) Combined with High Resolution Mass Spectrometer (HRMS)

Serge Auger*, Pierre Picard, Alex Birsan, Gregory Blachon, Jean Lacoursiere; Phytronix Technologies Inc, Quebec, Canada

Objectives: Toxicology laboratories generally use screening methods based on immunoreactivity. The selectivity of these automated instruments unfortunately allows for cross-interference caused by similar drugs. The cost of the reactants (enzymes or antibodies) could also amount to be very expensive. The high throughput Laser Diode Thermal Desorption (LDTD) ion source coupled with a high resolution mass spectrometer TripleTOF-5600 System (HRMS) constitutes an ultra-fast screening system for small molecules in urine. In this application we demonstrate how 34 drugs of abuse are quantified simultaneously in 7 seconds per sample.

Materials and Methods: The following drugs were spiked in urine at two times the cut off value used in typical screening procedure: Fentanyl; Norfentanyl; Propoxyfene; Norpropoxyfene; Methadone; EDDP; Codeine; Morphine; Oxycodone; Oxymorphone; Diazepam; Estazolam; Hydroxyalprazolam; Hydroxyethylflurazepam; Hydroxymidazolam; Hydroxytriazolam; Lorazepam; Nordiazepam; Oxazepam; Temazepam; 7-aminoclonazepam; 7-aminoflunitrazepam; Chlordiazepoxide; Meparidine; Normeperidine; THC-COOH; amobarbital; butalbital; Phenobarbital; Secobarbital; JWH-018 5-OH; JWH-073 4-OH; JWH-018 5-COOH; JWH-073 4-COOH. The urine samples are diluted two-fold in order to obtain the cut off concentration and a second dilution is done to obtain 50% of the cut off. The basic and neutral drugs are extracted using the following conditions: 50 µL urine sample, 50 µL internal standard in NaOH (0.2N) solution and 200 µL Ethyl acetate. The acid drugs are extracted using the following condition: 50 µL urine sample, 50 µL internal standard in HCl (0.2N) solution and 200 µL 1-Chlorobutane. After mixture and phase separation, 4 µL of organic phase is spotted onto the individual wells of a specially constructed 96-well plate.

Results: LDTD-HRMS System operated in TOF mode allows measurement of all compounds into the extract that vaporize and ionize. The system scans was set to a mass range between 100 to 900 amu. Extracting the signal in a mass window of 10 ppm gives sufficient specificity to quantify molecules and ovoid interference from compound of same nominal mass. The APCI ionization performed in positive and negative mode covers the properties of the set of molecules targeted. Analysis of the 34 spiked drugs in a urine sample needs desorption of 2 wells, positive and negative ionization mode. Each analysis has duration of seven seconds. All compounds give linear response and the sensitivity obtained largely exceed the required cut-off value.

Conclusions: The LDTD technology combined with a high resolution system; allows for a robust drug screening in spiked urine samples with a range well below their cut off values and a sample-to-sample run time of seven seconds.

Keywords: High Throughput, LDTD-High Resolution MS/MS, Drug Screening

P39

Ultra-Fast Online SPE/MS/MS Confirmation Analysis of THCCOOH for Forensic Toxicology

Michelle V. Romm*[1], Lawrence J. Andrade[2], Matthew Woodcock[2], Vaughn P. Miller[1], William A. LaMarr[1]; [1]Agilent Technologies Inc., Wakefield, MA, USA, [2]Dominion Diagnostics, North Kingstown, RI, USA

Introduction: Forensic drug testing has traditionally utilized GC/MS and more recently LC/MS as the analytical method of detection. Steady increases in the need for greater analytical capacity and throughput have placed demands on traditional technologies. We evaluated the ability of an ultra-fast SPE/MS/MS system to accurately measure 11-nor-9-delta-9-tetrahydrocannabinol (THCCOOH; the major metabolite of marijuana) in urine with much faster sample cycle times (under 15 seconds per sample) and similar analytical results compared to LC/MS/MS assays.

Objective: Cross validate the analysis of THCCOOH between ultra-fast SPE/MS/MS and LC/MS/MS.

Methods: Mass spectrometry and SPE methods were optimized for THCCOOH and its respective deuterated internal standard on a High-throughput RapidFire Mass Spectrometry System interfaced to a QqQ. Drug-free urine was spiked with THCCOOH, diluted, and injected for analysis. Human urine samples underwent base hydrolysis and off-line SPE preparation prior to SPE/MS/MS analysis. Sample cycle times for SPE/MS/MS were under 15 seconds per sample. Data analysis was performed using RapidFire Integrator software.

Results: THCCOOH standard curves were prepared in a wide dynamic range. THCCOOH had excellent linearity within its measured range of 5-5000 ng/ml with a R^2 value greater than 0.995. Intra and interday accuracies were within 10% and intra and interday coefficients within 5%. Carryover was assessed and found to be <5% of the lowest point of the standard curve. Blinded human samples were evaluated to further verify the SPE/MS/MS method and the results were compared to LC/MS/MS data. The cross validation correlation for blinded human samples which contained a variety of THCCOOH concentrations showed an R^2 value of 0.984 with the following equation: y = 1.0481x -0.321.

Conclusions: Based on these results, THCCOOH can be accurately and precisely confirmed in urine using ultra-fast SPE/MS/MS at rates of <15 seconds per sample and sensitivities at low ng/ml. This methodology is capable of throughputs of >240 samples per hour.

Keywords: THCCOOH, SPE/MS/MS

P40

More Bang for Your Buck - An Alternative Approach to Blood and Tissue Screening That Saves Time and Money

M. Elizabeth Zaney*, Diane M. Boland, George W. Hime; Miami-Dade County Medical Examiner Department, Miami, FL, USA

Background/Introduction: The Toxicology Laboratory at the Miami-Dade Medical Examiner Department recently changed the blood and tissue screening methodology from a multi-step liquid-liquid extraction (LLE) of basic drugs; to a dual-elution solid phase extraction (SPE) of acidic/neutral and basic drugs. This was done to achieve a more cost effective; comprehensive blood drug screen; which utilizes smaller solvent volume and reduces sample preparation time.

Objective: The objective is to present data comparing the previously utilized LLE procedure to the newly implemented automated SPE method. Examples will include spiked controls, proficiency samples and postmortem cases.

Methods:
The LLE procedure (described by Kopjak and Finkle et. al in JAT 1979) applies strictly to the extraction of basic drugs from 1 mL of sample. The extract is then analyzed by dual column GC-TSD. The SPE method is a modified version of United Chemical Technologies Procedure Code: DRB200DAUZ120392 using UCT Clean Screen® cartridges and an automated Zymark Rapid Trace® system. The procedure uses 1 mL sample volume yet yields two distinct fractions. The acidic/neutral extract is submitted for analysis by dual column GC-FID, and the basic extract is analyzed by GC-TSD. GC-Ion Trap/MS is performed to confirm any positive findings.

Results: The SPE method detected all 113 spiked control drugs and showed improved recovery for certain drugs, particularly the sympathomimetic amines and benzodiazepines. Co-elution of doxylamine and etomidate with caffeine was prevented since caffeine now elutes in the acidic/neutral extract. The improved detection of ephedrine; in addition to the detection of acetaminophen in the acidic/neutral extract was noted in two separate proficiency samples in which these drugs were missed when screened using the former LLE method. Screening of postmortem case samples utilizing the SPE method has led to detection of drugs in the acidic/neutral extract such as propofol, topiramate, levetiracetam, acetaminophen and valproic acid which would have previously been missed. Newer drugs detected in the basic extracts include BZP, TFMPP, 5MeoDIPT, Methylone and MDPV, which could have been missed due to decreased recovery by LLE. In addition heroin, 6-MAM, morphine and benzoylecgonine were detected in the initial GC/MS screening; as opposed to having to be specifically targeted in other confirmatory assays.

Discussion: With the constant evolution of designer drugs, it is important for laboratories to respond and adapt accordingly, even though funding for consumables and staff may be limited. By adopting an SPE protocol, the laboratory is now equipped to screen for a variety of tryptamines, as well as the components of the ever-so-popular bath salts. Additionally, the laboratory has become more efficient due to the reduction in solvent usage and sample preparation time. Other advantages include safety improvements and prevention of errors from multi-step procedures.

Conclusion: More information is obtained from the same sample volume via the dual elution, which provides a much more comprehensive screen.

Keywords: Solid Phase Extraction, Comprehensive Screening, Basic Drugs

P41

Screening for Drugs of Abuse in Forensic Toxicology Using an Ultra-Fast Online SPE/MS/MS System

Nikunj R. Parikh*, Kari Schlicht, Michelle V. Romm, Vaughn P. Miller, and William A. LaMarr; Agilent Technologies Inc., Wakefield, MA, USA

Introduction: Forensic drug screening is extensively used by Law Enforcement officials, employers and pathologists today. Traditionally the screening for these drugs involves analysis by immunoassays followed by a confirmatory test by GC/MS detection and more recently by LC/MS assays. The steady increase in sample size has created a bottleneck in screening for these drugs across different classes and longer turnaround times for confirmatory tests. While immunoassays are convenient and well established, they are not as sensitive or specific as LC/MS which leads to many false negatives and positives. In the present study, we evaluated the ability of an ultra-fast SPE/MS/MS system to screen across different classes of drugs of abuse in urine while approaching the sensitivity and accuracy of LC/MS and maintaining the speed and efficiency of a screen (sample cycle times <15 seconds per sample).

Objective: Validate SPE/MS/MS as a screening tool for the analysis of drugs of abuse.

Methods: Mass spectrometry and SPE methods were optimized separately for panels across drug classes; with their respective deuterated internal standards on a High-throughput RapidFire Mass Spectrometry System interfaced to a QqQ. Drug-free urine was spiked with each drug metabolite individually and as a panel for simultaneous analysis, diluted, and injected for analysis. Sample cycle times for all compounds were under 15 seconds per sample. Data analysis was performed using RapidFire Integrator software.

Results: Standard curves for each class of drugs were prepared in a wide dynamic range. Each curve had excellent linearity within its measured range; for example: benzoylecgonine within 31-4000 ng/ml and a panel of 5 benzodiazepines analyzed simultaneously were within 25-25;000 ng/ml with R^2 values greater than 0.995. Intraday accuracies were within 15% and intraday coefficient of variation values were all less than 5% for concentrations within the measured range. Blinded human samples were evaluated to further verify the SPE/MS/MS methods and the results were compared to LC/MS/MS data. The SPE/MS/MS system accurately measured positive and negative screening results for each analyte.

Conclusions: Based on these results, drugs of abuse including benzodiazepines and benzoylecgonine can be accurately and precisely screened in urine using ultra-fast SPE/MS/MS at rates of <15 seconds per sample and sensitivities at low ng/ml prior to confirmation analysis by LC/MS/MS. This methodology is capable of throughputs of >240 samples per hour.

Keywords: Drugs of Abuse, SPE/MS/MS, Forensic Toxicology

P42

A New Supported Liquid Extraction (SLE) Strategy with Liquid Chromatography Mass Spectrometry Detection for the Determination of JWH018, JWH073, CP47, 497, CP47, 497-C8, JWH200, JWH019, HU210 and JWH250 in Blood

Geraldine Dowling*[1], Lee Williams[2], Rhys Jones[1], Liam Regan[1]; [1]The State Laboratory, Backweston Laboratory Complex, Young's Cross, Celbridge, Co. Kildare, Ireland, [2]Biotage GB Limited, Ystrad Mynach, Hengoed, UK

Background/Introduction: The United States Drug Enforcement Administration (DEA) has controlled the following synthetic cannabinoid compounds JWH018; JWH073; CP47,497; JWH200 and cannabicyclohexanol since 2011. The compounds mimic the effects of cannabis but they have the potential to be extremely harmful and therefore pose an impending hazard to public safety. In early 2012 the DEA extended control of these compounds for another six months. Therefore in forensic applications and in order to identify hazards to public health internationally there is a requirement for new analytical strategies to detect these substances in blood.

Objective: The aim of this study was to develop a rapid analytical strategy utilizing SLE for synthetic cannabinoid compounds with detection by liquid chromatography hybrid triple quadrupole linear ion trap (QTRAP) mass spectrometry.

Method: Blood samples were passed through SLE columns (Biotage). Sample aliquots were analysed by hybrid triple quadrupole linear ion trap (QTRAP) mass spectrometry (AB Sciex). Multiple reaction monitoring (MRM) as survey scan and an enhanced product ion (EPI) scan as dependent scan were performed in an information-dependent acquisition (IDA) experiment in positive mode. Multiple reaction monitoring (MRM) was performed in negative mode. Drug identification and confirmation was carried out by library search with a developed inhouse MS/MS library based on EPI spectra at a collision energy spread of 50 ±15 in positive mode and MRM ratios in negative mode. The method was validated in blood according to the criteria defined in Commission Decision 2002/657/EC. At least two MRM transitions for each substance were monitored in addition to EPI spectra and deuterated analogues of analytes were used as internal standards for quantitation where possible. The validation parameters of linearity; precision; recovery; specificity; decision limit (CC alpha) and detection capability (CC beta) were determined.

Results: The proposed method showed good specificity and sensitivity for the selected spice compounds. Calibration curves were linear in the region of 2-100 ng/mL for JWH018; JWH073; CP47, 497; CP47,497-C8; JWH200; JWH019 and HU210. Quantitation was poor for JWH250. The substances could easily be detected at the lowest concentration on the matrix matched curve in blood at a concentration of 2 ng/mL routinely.

Discussion/Conclusion: To the best of our knowledge there are no published data regarding the use of SLE for the extraction of synthetic cannabinoid compounds from blood. The fast sample preparation turnaround time and simplicity of the SLE procedure and LC-MS detection could be useful in postmortem forensic toxicology or in emergency intoxication cases and in public health monitoring programmes. The data in the poster highlights a simple extraction and detection protocol for the determination of the synthetic cannabinoids in blood.

Keywords: Designer Drugs, Synthetic Cannabinoids, Supported Liquid Extraction, Liquid Chromatography Mass Spectrometry

P43

Extraction of Low Levels of Barbiturates from Human Urine Using Supported Liquid Extraction (SLE) Prior to GC-MS Analysis

Adam Senior[1], **Rhys Jones*[1]**, Lee Williams[1], Geoff Davies[1], Helen Lodder[1], Steve Jordan[1], Victor Vandell[2], Gavin Jones[1], Claire Desbrow[1]; [1]Biotage GB, Ystrad Mynach, Hengoed, UK, [2]Biotage LLC, Charlotte, NC, USA

Background/Introduction: Barbiturates are a group of compounds which were developed and primarily used in the early part of the 20th Century to alleviate anxiety and nervousness. Today's anti-anxiety therapy is more widely done with alternative drugs which have less potential for CNS problems and fatal overdose. Despite this, acute barbiturate poisoning is still reported world-wide. The shorter-acting barbiturates are favoured by some recreational users and this class of compounds also has relevance in post-mortem toxicology, and in cases of accidental or intentional suicide.

Objective: The objective was to develop a GC-MS assay for the determination of barbiturates from urine matrix using Supported Liquid Extraction (SLE). The SLE extraction mechanism is very efficient, delivering higher analyte recoveries and cleaner extracts than equivalent LLE methods.

Methods: Extraction conditions were evaluated using 0.5 mL matrix spiked with 7 barbiturates and pre-treated (1:1, v/v dilution) with 100mM ammonium acetate pH 5 to provide pH control. Sample hydrolysis was performed with beta-glucuronidase (type H-3 ex. H. pomatia). Sample preparation of 1 mL of the pre treated urine was performed using SLE with ISOLUTE® SLE+ columns. Extraction was investigated using 5 mL volumes of various water immiscible extraction solvents and solvent combinations. Samples were analyzed using an Agilent 7890 GC with a 5975 MSD. Chromatography was performed on a Zebron ZB-35 capillary column (30 m x 0.25 mm, 0.25 µm df) using 2 mL min^{-1} helium (constant flow) as the carrier. The inlet was operated at 150 °C in splitless mode. Samples were derivatized using in-port flash alkylation with 0.2 M TMAH (1 µL to 1 µL sample). The oven was programmed from 120 °C at 15 °C min^{-1} to 290 °C for 2 min giving a 13 minute run time. Positive ions were acquired using electron ionization operated in SIM mode. Ions were acquired in six SIM groups with a dwell time of 100 ms per ion with one quantitative ion and two qualifying ions for each analyte.

Results: This study investigated the extraction of the following barbiturates from human urine: butalbarbital, butabarbital, amobarbital, pentobarbital, secobarbital, hexobarbital and phenobarbital. For method development purposes the initial spike concentration used was 100 ng mL^{-1} when extracting 0.5 mL matrix. No analyte losses were seen on blow-down and reconstitution. In order to vary selectivity, the extraction solvents evaluated included: ethyl acetate, MTBE, diethyl ether, DCM and hexane. Recoveries were 95% to 105% using: DCM, with RSDs below 10%, diethyl ether proved a useful non-halogenated alternative. No detrimental effects were seen on enzymatic hydrolysis (recoveries 101% to 112%). Robustness of the extraction method was assessed using volunteer urine from 4 sources and gave close agreement. LOQs were determined to be 10 ng mL^{-1} with S/N of 14:1 or greater and RSDs of 6% or less.

Conclusion/Discussion: Barbiturates are lipophilic molecules with high LogP values and are therefore amenable to partitioning into the organic extraction solvents. This poster shows the applicability of ISOLUTE SLE+ to extract low levels of barbiturates from urine demonstrating reproducible extraction efficiencies and correspondingly low RSDs.

Keywords: Barbiturates, Supported Liquid Extraction, GC-MS

P44

Extraction of Benzodiazepines from Human Urine Using Supported Liquid Extraction (SLE) Prior to GC-MS Analysis

Adam Senior[1], **Rhys Jones*[1]**, Lee Williams[1], Geoff Davies[1], Helen Lodder[1], Steve Jordan[1], Victor Vandell[2], Gavin Jones[1], Claire Desbrow[1]; [1]Biotage GB, Ystrad Mynach, Hengoed, UK, [2]Biotage LLC, Charlotte, NC, USA

Background/Introduction: Benzodiazepines are psychoactive drugs which bind to GABA receptor complexes leading to sedative and anxiolytic effects. Non-medical use of benzodiazepines gives an effect similar to alcohol abuse and is often associated with other drugs of abuse. Sample preparation of urine samples is recommended to counteract any matrix effects that may be seen during the analysis of these samples.

Objective: The objective was to develop a GC-MS assay for the determination of free benzodiazepines using Supported Liquid Extraction (SLE). The SLE extraction mechanism is very efficient, delivering higher analyte recoveries and cleaner extracts than equivalent LLE methods.

Methods: Extraction conditions were evaluated using 0.5 mL matrix pre-treated (1:1; v/v dilution) with 100 mM ammonium acetate pH 5 to provide pH control. Samples were hydrolyzed using 25 µL (approx. 2250U) beta-glucuronidase (type H-3, ex. H. pomatia) for 2 hours at 60 °C. The sample was adjusted to approximately pH 8 using 10 µL 25% ammonia (conc). Sample preparation of 1 mL of the pre treated urine spiked with 18 benzodiazepines and metabolites was performed using SLE with ISOLUTE® SLE+ columns. Extraction was investigated using 5 mL volumes of various water immiscible extraction solvents and solvent combinations. Samples were derivatized prior to analysis 1:1 with MTBSTFA + 1% TBDMCS. Samples were analyzed using an Agilent 7890 GC with a 5975 MSD. Chromatography was performed on an Agilent HP 5 ms capillary column (30 m x 0.25 mm; 0.25 µm df) using 1.2 mL min^{-1} helium (constant flow) as the carrier. The inlet was operated at 250 °C, 2 µL injections were made in split mode with a 12.5:1 ratio. The oven was programmed at 50 °C for 0.5 min; ramp 1 at 25 °C min^{-1} to 150 °C for 0 min; ramp 2 at 10 °C min^{-1} to 325 °C for 6 min giving a 28 minute run time. Positive ions were acquired using electron ionization operated in SIM mode. Ions were acquired in 10 SIM groups with a dwell time of between 5 ms and 25 ms per ion with one quantitative ion and two qualifying ions for each of the 18 benzodiazepine analytes.

Results: This study investigated the extraction of the following benzodiazepines and metabolites from human urine: diazepam, nordiazepam, flunitrazepam, midazolam, 7-aminoflunitrazepam, bromazepam, oxazepam, nitrazepam, flurazepam, temazepam, 7-aminoclonazepam, lorazepam, hydroxyethylflurazepam, estazolam, alprazolam, triazolam, alpha-hydroxyalprazolam and alpha-hydroxytriazolam. For method development purposes the initial spike concentration used was 100 ng mL^{-1} when extracting 0.5 mL matrix. Because of the need to hydrolyze samples prior to analysis, variation in pre-treatment was not possible. In order to vary selectivity, the extraction solvents evaluated included: ethyl acetate, MTBE, DCM and 5% IPA in DCM. The data shows recoveries in excess of 80% for most analytes. LOQs were at or below NIDA cut-offs.

Conclusion/Discussion: Benzodiazepines are lipophilic molecules with LogP values above one and are therefore amenable to partitioning into the organic extraction solvents. This poster shows the applicability of ISOLUTE SLE+ to extract benzodiazepines from urine demonstrating reproducible extraction efficiencies and corresponding RSDs below 10%.

Keywords: Benzodiazepines, Supported Liquid Extraction, UPLC-MS/MS

P45

Evaluation of SPE Strategies for the Simultaneous Extraction of Ethyl Glucuronide (EtG) and Ethyl Sulfate (EtS) Prior to LC-MS/MS Analysis

Lee Williams[1], Rhys Jones[1], Adam Senior[1], **Geoff Davies*[1]**, Helen Lodder[1], Steve Jordan[1], Victor Vandell[2], Gavin Jones[1], Claire Desbrow[1]; [1]Biotage GB Limited, Ystrad Mynach, Hengoed, UK, [2]Biotage, LLC, Charlotte, NC, USA

Introduction: Ethyl glucuronide and ethyl sulfate are specific metabolites formed within the body after the ingestion of alcohol. In forensic toxicology; when tested together they provide more specific evidence of alcohol intake. The simultaneous extraction of EtG and EtS is complicated due to the lack of hydrophobicity on these molecules along with the fact that EtS has a strongly acidic functionality while EtG has a weakly acidic moiety. The other interfering factor is the ionic nature of the matrix which can suppress interaction when using anion exchange SPE protocols. As a result dilute and shoot approaches are often employed prior to LC-MS/MS analysis.

Objective: This poster will demonstrate SPE sample preparation strategies for the simultaneous extraction of both ethyl glucuronide and ethyl sulphate from urine, allowing cleaner extraction protocols compared to traditional dilute and shoot approaches.

Method: Blank human urine was spiked with EtG/EtS at various concentrations and extracted using multiple forms of SPE. Initial method development was performed in the 25mg 96-Well plate format extracting 50-100µL of urine at 200 ng/mL. Resin-based mixed-mode anion exchange was investigated using both strong and weak sorbents (EVOLUTE AX and WAX). All samples were analysed using a Waters 2795 liquid handling system coupled to a Quattro Ultima Pt triple quadrupole mass spectrometer. Negative ions were acquired using electrospray ionization operated in the MRM mode: EtG; 220.8 > 84.9 and 220.8 > 74.9; EtS; 124.8 > 96.8 and 124.8 > 79.8. Chromatography was performed using a Kinetex C18 (2.6 µ; 2.1 x 100 mm) with isocratic mobile phase conditions of 98/2; 0.1% formic acid aqueous and acetonitrile.

Results: Extraction results demonstrated that substantial analyte breakthrough was observed if any buffers were used in the pre-treatment or SPE column conditioning steps for both mixed-mode sorbents. Urine dilution factors were also an important consideration due to analyte breakthrough. Strong anion exchange shows high degree of suppression using standard aqueous based protocols. However; using modified protocols incorporating a polar aprotic solvent such as ACN; demonstrated better extract cleanliness. This strategy shows recoveries greater than 80% recovery for EtG but low EtS extraction due to strong retention of the sulfate group on the quaternary amine functionality of the SPE sorbent. Modification of the elution solvent with HCl instead of formic acid increased extraction recovery to greater than 70% for EtS. WAX protocols generally demonstrated good recovery for one analyte but not the other. Aqueous based methods demonstrated greater than 80% recoveries for EtS but recoveries less than 20% for EtG. When using an ACN protocol on the WAX sorbent as per the AX methodology, EtS retention was an issue. Further method modification of the wash and elution steps was required to increase recoveries. Full results will be shown in the final poster.

Conclusion: This poster provides mixed-mode resin-based SPE sample preparation strategies for the simultaneous extraction of EtG and EtS. The advanced cleanliness of SPE compared to dilute and shoot approaches can lead to improvements in precision and accuracy when performing method validation. SPE strategies also provide a concentration step during blow down resulting in lower achieved limits of quantitation and/or the use of lower matrix volumes.

Keywords: SPE, EtG, EtS, LC-MS/MS

P46
The Analysis of Bath Salts Using Solid Phase Extraction and GC-MS

S. Adams*, K. Evans, Tony Edge; Thermo Fisher Scientific, Bellefonte, PA, USA

Background: Bath Salts are synthetic drugs that are currently legal to purchase in many states within the US. With names such as White Rush; Blizzard; and Fine China. These substances are being sold to people looking to get a high comparable to drugs such as cocaine and methamphetamines. As well as the euphoric sensations that these compounds can give, there are also other symptoms such as paranoia, anxiety and hallucinations which are observed with users of these classes of drugs. Many US states now ban the sale and possession of bath salts, with more states considering legislation to make them illegal as well. When these synthetic drugs do become illegal, an analytical method will be needed to analyze and monitor trace levels of these compounds in humans.

Objective: This presentation looks at some of the known active ingredients in bath salts; e.g. mephedrone, methedrone, methylone, butylone, 4-methylethcathinone and methylenedioxypyrovalerone (MDPV). It will present a method used for the isolation and the quantification of these compounds from the original bath salt.

Methods: Two extraction procedures were developed. One procedure uses the Servo Total B SPE cartridge and the other uses the Servo+ Total B SPE cartridge. Once the samples had been extracted they were derivatized with MSTFA + 1% TMCS and then analyzed using GC-MS in SIM mode, monitoring three ions for each compound.

Results: Optimization of the extraction procedure determined the optimal load, wash and elution conditions for both types of SPE cartridge. The separation of the individual components was performed using GC-MS which was able to resolve chromatographically the six components. Extra peaks were also detected and these are believed to be derivatives of methylcathinone.

Conclusion: An effective method for the isolation of six common components found in bath salts was developed. The extraction was performed using the novel Servo and Servo+ Total B SPE cartridges. Optimization of the extraction process ensured that the cleanest extracts were obtained which were then analyzed using a GC-MS method. Analysis of some of the newest bath salts on the market includes active ingredients that are not controlled and are still legal to use.

Keywords: LCMSMS, SPE, Bath Salts

P47

Discrimination of Single and Repeated Amitriptyline Exposure in Skeletal Tissues Using Ultra-High Performance Liquid Chromatography

Heather M. Cornthwaite*, Caroline C. Betit, James H. Watterson; Department of Forensic Science, Department of Chemistry and Biochemistry, Laurentian University, Sudbury, Ontario, Canada

Introduction/Objectives:
Bone remains a poorly characterized matrix for toxicological analysis. The purpose of this work was to determine if two different patterns of amitriptyline exposure could be discriminated through measurement of amitriptyline (AMI) and nortriptyline (NORT) levels, or the ratio of levels of NORT and AMI (i.e.; NORT/AMI) in decomposed bone.

Methods: Twelve male Wistar rats were divided into two groups with five rats per dose condition and two drug-free control animals. The first group (ACU) received one amitriptyline injection (120 mg/kg i.p.) and were sacrificed by CO2 asphyxiation 15 minutes post-dose. The second group (repeated dose - REP) received two injections of amitriptyline (40 mg/kg each; i.p.) and were sacrificed 40 minutes after the second dose. Remains decomposed to skeleton outdoors in a natural setting (Northern Ontario) in secure cages for 3 weeks. Eight different bone types (humerus; ulna; radius; scapula; vertebrae; ribs; pelvis and femur) were recovered from each group. Bones were washed with methanol, phosphate buffer (PBS: 0.1 M; pH 6) and acetone (3 mL each) and left to air-dry overnight. Bones were then ground using a domestic grinder. Samples of each bone (0.2 g) were then extracted in methanol (10 mL; 72 hrs; 50 ^{o}C). Extracts were recovered, evaporated to dryness and reconstituted in 1ml PBS. Internal standard (Desipramine; DESI; 200 ng), 100 µL glacial acetic acid and acetonitrile:methanol (1:1; 3 mL) was added to each extract; followed by storage at -20 ^{o}C overnight. Following centrifugation, supernatants were evaporated to 1 ml; and diluted with 3 mL PBS. Diluted supernatants underwent SPE. Columns were conditioned with methanol (3 mL), water (3 mL) and PBS (3ml). Following sample loading, columns were washed with PBS (3ml) and 0.1 M acetic acid (3 mL), and dried (~ 5 in Hg; 5min). Columns were then washed with methanol (3 mL) and dried again under vacuum (~10 in Hg; 10 min). Basic compounds were eluted with 3% NH_4OH in 20:80 isopropanol:ethyl acetate (6 mL). Extracts were evaporated to dryness and reconstituted in 0.1% formic acid in 10:90 acetonitrile:water (500 µL). Samples were analyzed by Ultra-High Performance Liquid Chromatography with Photodiode Array Detection (UPLC-PDA). The wavelengths monitored were 240 nm for amitriptyline and nortriptyline; and 290 nm for desipramine. AMI and NORT levels were measured as the mass-normalized response ratios (RR/m). Drug levels and NORT/AMI ratios were compared using Student's t-test, significance attributed where $p < 0.05$.

Results: For all bones examined, levels (RR/m) of AMI or NORT did not differ significantly between exposure patterns. However, in all bones except rib, values of NORT/AMI differed significantly between exposure patterns ($p < 0.05$). When results were pooled across all bone types, neither AMI or NORT levels did not differ significantly ($p = 0.06$, 0.11, respectively). However, values of NORT/AMI differed significantly between exposure patterns ($p < 0.0001$).

Conclusions: Acute and repeated exposures of rats to amitriptyline were successfully discriminated by measurement of the ratio of levels of primary metabolite and parent drug (i.e.; AMI/NORT) in decomposed bone.

Keywords: Amitriptyline Exposure

P48

UPLC-MS/MS for the Screening, Confirmation and Quantification of 32 Drugs Illegally Added to Herbal/Dietary Supplements for the Enhancement of Male Sexual Performance

Salman Azimi[1], **Nayan Mistry*[2]**, Michelle Wood[2]; [1]Drug Quality Control Lab, Pharmacy & Drug Control Dept, Supreme Council of Health, Doha, Qatar, [2]Waters Corporation, Atlas Park, Wythenshawe, Manchester, UK

Introduction: The adulteration of herbal/dietary supplements with erectile dysfunction (ED) drugs and their analogues is reported worldwide and is an increasing problem(1,2). The sale of so-called 100%, 'all-natural' products has become a highly profitable business for online pharmacies, however these products can pose a serious threat to consumers owing to the undisclosed presence of approved/prescription drugs or the unknown safety and toxicity profile of unapproved ED drugs. Government authorities play a crucial role in the control of these products for the safety of human health.

Objective: The objective of the study was to develop an analytical procedure for the herbal/dietary products that are imported into Qatar and marketed to improve male sexual performance.

Method: A simple and rapid UPLC-MS/MS procedure for the analysis of 32 synthetic ED compounds in herbal supplements, without sample cleanup, is presented. A spectral library for the synthetic compounds and their analogues was generated from reference standards for automated routine sample screening. Full scan MS analysis was performed simultaneously in both positive and in high energy negative ESI modes; the latter function permitted the detection of new/unknown ED analogues by generation of common, high intensity fragment ions at m/z 232, 282 and 298. In addition a MS/MS method was developed for confirmation and quantification using two multiple reaction monitoring (MRM) transitions for each compound. This method was validated for three matrices: capsules/tablets/pills, honey and herbal drink. Calibration curves (0.2ng/mL - 1000ng/mL) were prepared in the presence and absence of matrix.

Results: The limit of quantification was 0.5ng/mL for 29 compounds based on a signal-to-noise ratio of ≥10:1 for both quantifier and qualifier ions and with %CV reported <11% for 32 compounds spiked in herbal matrices at 2ng/mL. The method was applied to 43 suspected dietary products which were imported into Qatar and were received from customs, herbal registration section and clearance section of health authorities during 2010/2011. A total of 18 products were found to be adulterated: 11 with sildenafil, two with thiodimethylsildenafil and five found to contain a combination of yohimbine, tadalafil, aminotadalafil, dimethylsildenafil and thiosildenafil.

Conclusion: A novel screening method has been established for the detection of known and unknown ED compounds and their analogues. The acquired data is automatically matched to a spectral library using ChromaLynx™ application manager. The high cone voltage in ESI- mode generates common ions and therefore identifying unknown analogues which help to ascertain its compound category. The MS/MS analysis provides a sensitive, selective, accurate assay that also demonstrates excellent linearity and increases confidence in results.

References:

(1) Tainted Sexual Enhancement Product, http://www.fda.gov/Drugs/ResourcesForYou/Consumers/BuyingUsingMedicineSafely/MedicationHealthFraud/ucm234539.htm

(2) Hidden Risks of Erectile Dysfunction "Treatments" Sold Online, http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm048386.htm

Keywords: UPLC-MS/MS, ED Analogues, Adulteration, Herbal/Dietary Supplements

P49

An Explanation of Lingering “Opiate" Deaths? Relative Concentration of Opiates in Medulla and Femoral Blood Following Lethal Intoxications

Claire K. Kaspar*, John Wyman, Kimberly Snell, Eric Lavins, Thomas Gilson; Cuyahoga County Regional Forensic Science Lab, Cuyahoga County Medical Examiner's Office, Cleveland, OH, USA

Introduction: Toxicologists and pathologists are sometimes confronted with difficult cases where the case history surrounding the deaths indicates an opiate overdose, but the postmortem blood levels measured are only in the therapeutic range. These deaths are generally referred to as “Lingering Deaths" and the decedent is thought to have reached a lethal level of intoxication but remains alive sufficiently long enough to metabolize and eliminate the high level of opiates from blood down to therapeutic concentrations. Death from opiate intoxication is most often a result of respiratory and cardiac depression with resultant pulmonary pathology producing increased lung weights. Neurological control of respiration and cardiac rhythm resides in the medulla oblongata. Cases selected for this study were chosen based on the suspicion of overdose indicated in the case history.

Objective: To uncover a possible explanation of lingering deaths, this study examined the relative concentration of opiates in femoral blood compared to opiate concentrations in the medulla oblongata.

Methods: Specimens were collected during autopsy and kept refrigerated until processed for analysis. Extraction of drugs was accomplished using solid phase extraction (UCT Clean Screen ZSDAU020) followed by derivatization with MSTFA (UCT). Analytes were separated, detected and quantitated by an Agilent GC/EI- MS in the SIM mode using a Restek Rxi-5ms, 30 m X 0.25 mm i.d., 0.25 µm film thickness, analytical column.

Results: As is shown in Table 1, the average opiate concentrations for morphine, codeine and 6-acetylmorphine were higher in medulla than in blood.

Table 1: Distribution of Opiates in Blood and Brain (ng/mL):

Sample	Blood Morphine	Brain Morphine	Blood Codeine	Brain Codeine	Blood 6-AM	Brain 6-AM	Br:Bld Morphine	Br:Bld Codeine	Br:Bld 6-AM
Average	136	189	16	50	15	51.2	1.39	3.07	3.31

Five of the twenty cases examined had morphine concentrations higher in blood than brain. However, examination of the case histories revealed that these cases were acute exposures with drug paraphernalia still present at the death scene in three of the five cases. It is likely that the heroin did not have sufficient time to distribute to brain before death occurred. In all cases, the codeine and 6-acetylmorphine levels in medulla were higher than that determined for blood. Examination of the brain:blood ratio for the three analytes demonstrated an increasing ratio from morphine, to codeine, to 6-AM; which directly corresponds to the relative lipophilicity of these analytes. Other opioids investigated (oxycodone and hydrocodone) demonstrated the same preferential distribution to medulla relative to femoral blood.

Conclusion: The preferential distribution of opiates to medulla (site of respiratory control) suggest that lingering opiate deaths may be explained, at least in part, because of higher relative concentrations of drug in brain, compared to femoral blood.

Keywords: Opiates, Medulla, Lingering Deaths

P50

Advanced Automated Library Searching for Compound Identification in Forensic Toxicology Samples

Jeffery Rivera*[1], Janna Anichina[1], Michael Jarvis[1], Adrian Taylor[1], Rolf Kern [2], Kara L. Lynch[3]; [1]AB SCIEX, Concord, Ontario, Canada, [2]AB SCIEX, Foster City, CA, USA, [3]Chemistry and Toxicology Laboratory, San Francisco General Hospital, San Francisco, CA, USA

Introduction: (Not for Use in Diagnostic Procedures)LC-MS/MS utilization in forensic toxicology screening for drugs and drug metabolites has become increasingly popular due to the selectivity, sensitivity and the speed of LC-MS/MS analysis. MS/MS confirmation with automated searching against available spectral libraries has proven to add a superior level of confidence to the compound identification. One of the key factors of the complete solution for forensic toxicology screening is automation of the library searching with the advanced capability to dynamically review the acquired data. Solutions need to be accurate and robust. The ability to search multiple libraries, create subsets of libraries, adjust and refine search parameters as well as re-search acquired data provides the user with the substantial flexibility. Setting mass tolerance, intensity thresholds and searching multiple collision energies enhance the data under revision. This can improve overall data quality and throughput.

Objective: The objective of this study was to demonstrate the advantages of the new automated library searching approach in improving typical forensic toxicology screening workflows.

Methods: Samples were analyzed using generic sample preparation procedures with two AB SCIEX LC-MS systems: a hybrid linear ion trap-triple quadrupole system and a hybrid quadrupole-time-of-flight instrument. The MS/MS measurements were performed using Collision Energy Spread (CES) feature which ensures the detection of the fragment ions generated in low-; medium- and high-collision energy regimes. All the collected MS/MS spectra were searched against an AB SCIEX Forensic Drug Spectral Library comprised of over 1250 compounds. The data processing was performed with the new AB SCIEX prototype library searching tool equipped with two library search algorithms.

Results: The the processing of the data specifically acquired in different experimental set-ups. The ion trap screening data were collected in three screening workflows that consisted of several looped experiments as follows:

I. Multi-targeted Screening
(i) MRM detection of 300 analytes with the Scheduled MRM ™ algorithm
(ii) EPI dependent scans set to collect MS/MS fragmentation spectra for the targets detected in experiment (i)
II. General Unknown Screening
(i) Enhanced Mass Spectrum (EMS) monitoring for the detection of the unknown analytes
(ii) EPI dependent scans set to collect MS/MS fragmentationspectra for the unknowns detected in experiment (i)
III. Combined Multi-targeted and unknown screening
(i) MRM detection of 300 analytes with the Scheduled MRM ™ algorithm
(ii) EMS monitoring for the detection of the unknown analytes.
(iii) EPI dependent scans set to collect MS/MS fragmentation spectra for the targets identified in experiment (i) and unknowns identified in experiment (ii)

We have also processed the hybrid quadrupole-time-of-flight data collected using a TOF-MS survey scan with IDA-triggering of up to 20 product ion scans. Both targeted and unknown drugs and metabolites were identified in selected patient samples with high level of confidence (based on the values of purity, fit and reverse fit).

Conclusion: Utilization of the new advanced automated library searching with the capability to dynamically review the collected MS/MS information has been demonstrated to substantially improve the “data mining" process and provide an elegant solution to automated processing of the forensic toxicology screening data.

Keywords: Mass Spectrometry, Library Searching, Quadrupole Time-of-Flight, Hybrid Linear Ion Trap-Triple Quadrupole

P51

Effects of Aerobic vs. Anaerobic Metabolism by Escherichia Coli on Cocaine and Fentanyl

Stephanie Martindale*[1], Suzanne Bell[1], Robert Powers[2], Sue Bae[3]; [1]West Virginia University, Morgantown, WV, USA, [2]University of New Haven, New Haven, CT, USA, [3]Edgewood Chemical Biological Center at Aberdeen Proving Grounds, Gunpowder, MD, USA

Introduction: Anaerobic bacteria and microbes native to the human body are active in the post-mortem decomposition process, especially during the putrefaction and decaying phases. After death, these species may cause biotransformations that affect the ratio of compound to metabolite concentration within the human body. To date, such post-mortem changes are rarely considered. This may lead to discrepancies in compound identification, which could ultimately influence information concerning time-since-death estimations.

Objective: This research aims to explore the potential of using enteric bacterial species to determine whether such biotransformations occur, how they occur, to analyze the types of anaerobic bacterial metabolites produced, and to compare these metabolites to those produced by aerobic metabolism. The purpose of this study was to establish protocol for growing, inoculating, and dosing the bacteria in both aerobic and anaerobic conditions, and to design a qualitative detection method to compare the types of bacterial metabolites produced.

Methods: These experiments focused on *Escherichia coli* (*E. coli*), a Gram-negative, facultative anaerobe, which is native to and prevalent in the intestinal tract. The *E. coli* specimen was a standard obtained from human feces. In addition, two drugs of forensic importance (and their metabolites) were chosen as the compounds of interest, which include cocaine (COC): anhydroecgonine methyl ester (AEME), benzoylecgonine (BE), cocaethylene (CE), ecgonine methyl ester (EME), m-hydroxycocaine (mHC), and norcocaine (NC) and fentanyl (FE): norfentanyl (NF). The bacteria were grown aerobically in ambient oxygen conditions and anaerobically using a certified anaerobic system. Single, pure colonies were isolated on tryptic soy agar plates and used in the inoculation of tryptic soy broth for the metabolic analysis studies. Broth samples were spiked with one of the selected parent drugs, inoculated with 250µL of bacterial broth, and incubated for 72 hours at 37 C. Analytes were extracted from media using solid phase extraction and then and analyzed using LC/MS/MS. Chromatographic separation and identification of the analytes were achieved using a core shell C18 (2.6µm; 2.1 x 30mm) column, and the mass spectrometer was operated in positive mode with electrospray ionization.

Results: A qualitative method was established for each drug and its respective metabolites using a 0.1µg/L standard drug solution. Analytes were detected via the following characteristic MRM transitions: COC - 304.1, 182.1, 150.1, 82.1; AEME - 182.1, 118.1; BE - 290.1, 168.1, 150.0, 82.1; CE - 318.1, 196.1, 150.1; EME - 200.2, 182.1; mHC - 320.1, 182.1; NC - 290.3, 168.1; and FE - 337.0, 188.4; NF - 233.0, 84.3. Cocaine D3 and fentanyl D5 were used as internal standards.

Conclusion: The use of enteric bacterial species and their roles in anaerobic biotransformations and metabolism has not, to our knowledge, been investigated. We recognize that the actual decomposition process is chaotic and each case unique; however, the goal is to start simply and then expand upon that base of knowledge. In the future, we aim to quantify the drug compound to bacterial metabolites ratio, validate the method, and extend this technique to explore other homeland security threats including additional drugs of abuse, chemical warfare agents, and explosives.

Keywords: Anaerobic Bacteria, Metabolism, LC/MS/MS

P52

Validated LC-MS/MS Assay for the Quantitative Determination of Selected Drugs of Abuse and their Pyrolytic Products in Urine and Plasma

Lucy S. Oldfield*, Suzanne C. Bell; West Virginia University, Morgantown, WV, USA

Introduction: Recent work involving pyrolysis of common drugs of abuse has shown evidence of distinct products. Presence of these pyrolytic products in biological matrices may be an indication of smoking abuse; however, common assays do not include pyrolytic products or their associated metabolites. Anhydroecgonine (AECG) has been previously reported as a pyrolytic product of cocaine. Propionanilide (PROP) has been shown, in work carried out in this laboratory, as stable pyrolytic product of fentanyl. While it would be possible to develop a gas chromatography mass spectrometry assay for detection of these pyrolytic markers, liquid chromatography tandem mass spectrometry (LC-MS/MS) is a more attractive technique offering greater selectivity and sensitivity.

Objective: The current study involves the validation of a LC-MS/MS method for analysis of cocaine, fentanyl and their major pyrolytic products and relevant metabolites in urine and plasma.

Methods: Calibration and quality control urine and plasma samples were prepared from pooled specimens. Urine was prepared by adding standard and internal standard (IS) solutions to 1 mL of specimen. Samples were diluted 1:1 with water followed by cold acetonitrile, centrifuged and the supernatant evaporated under N_2. Samples were reconstituted in mobile phase and subjected to LC-MS/MS analysis. Plasma was spiked with standard and IS solutions and extracted using mixed mode strong cationic exchange solid phase extraction. Extracts were evaporated under N_2 and reconstituted in 100 µL mobile phase for analysis. Separation was performed by gradient RP liquid chromatography using a core shell C18-XD column (2.6 µm particle size, 30 x 2.1 mm) and a mobile phase consisting of 5 mM ammonium formate with 0.1% formic acid and methanol with 0.1% formic acid at a flow rate of 0.45 mL/min. Mass analysis was performed with electrospray ionization (ESI) in positive mode using multiple reaction monitoring (MRM). A minimum of two characteristic MRM transitions for each compound (ion ratio ± 15%) were selected with a total run time of 5.5 minutes. Quantitative transitions were chosen as: cocaine 304.3/182.3 m/z, norcocaine 290.3/168.2 m/z, ecgonine 186.2/168.2 m/z, AEME 182.1/91.1 m/z, AECG 168.1/91.1 m/z, fentanyl 337.3/105.2 m/z, norfentanyl 233.3/84.2 m/z, DESP 281.3/105.1 m/z, PROP 150.2/94.2 m/z. IS transitions were: cocaine-d3 307.2/185.2 m/z and norfentanyl-d5 238.2/84.0 m/z.

Results: The following parameters were used to evaluate the assay; selectivity, matrix effects, recovery, linearity and both within and between-run bias and precision. Recovery and matrix effects were considered acceptable (< 15% variation). The assay was linear ($r \geq 0.99$) from 0.1 or 1- 250 ng/mL (compound/matrix dependent). Limit of detection was determined as 0.05 - 1 ng/mL. Limit of quantitation (< 20% precision, ± 20% accuracy and ion ratio ± 15%) was 0.5 – 5 ng/mL. Within and between-run precision and bias were less than 15% for all components (20% at LOQ).

Conclusion: This study demonstrates the potential use of AECG and PROP as markers for smoked cocaine and fentanyl respectively. A sensitive LC-MS/MS method for the identification of both pyrolytic products and their metabolites has been presented. This method could be adapted for analysis of other pyrolytic products.

Keywords: Pyrolysis, Biomarkers, LC/MS/MS

P53

An Outbreak of Heroin Related Deaths in a Major Midwest Metropolitan City - The Cleveland Experience Over a 6-Year Period, (2006-2011)

Claire K. Kaspar*[1], Eric S. Lavins[1], Rindi N. Norris[2], John F. Wyman[1], William D. Alexy[1], Thomas P. Gilson[1];
[1]Cuyahoga County Regional Forensic Science Laboratory, Toxicology Department, Cuyahoga County Medical Examiner's Office, Cleveland, OH, USA, [2]University of Toledo, OH, USA

Introduction: The Toxicology Department at The Cuyahoga County Medical Examiner's Office (CCMEO) observed a dramatic increase in heroin-positive cases over the past year and a half. Statistical data generated from postmortem cases received at the CCMEO over the past six years indicate there were 52 heroin-related deaths in Cuyahoga County in 2006 (3.98 deaths per 100,000 people), in contrast to 105 heroin-related deaths in 2011 (8.20 deaths per 100,000 people). This represents a two-fold increase in heroin fatalities during the six-year study period.

Objective: To present the incidence of heroin deaths from 2006 to 2011 in Cleveland, Ohio (population 396,815) and Cuyahoga County as a whole (population 1,280,122). This information may help curb this alarming trend by creating awareness for public health officials, law enforcement, and other agencies dealing with drugs of abuse issues.

Methods: In the 6-year time frame there were 16,344 autopsies or death investigations performed at CCMEO with 7,965 requests for full Toxicology analysis. Results for this study were obtained by a statistical package which is part of the Toxicology Department's Pathways© program. This database is a centrally managed, interactive, real-time, data sharing environment program that allows the intra- and interdepartmental access to common data. The CCMEO Toxicology Department performed testing on the decedents by running Volatiles, a 13-panel (ELISA) screen, Color tests, and Basic and Acidic/Neutral drug analysis. The Opiate Confirmation analysis utilized UCT (United Chemical Technologies) Clean Screen ZSDAU020 extraction columns from a previously published UCT method for Opiates. Analysis was performed by GC/MS operated in the SIM mode. A multipoint calibration was utilized. Analytes were separated, detected, and quantitated by an Agilent GC/EI- MS with a Restek Rxi-5ms capillary column.

Results: There has been a dramatic epidemiological change in the number of heroin-related deaths in greater metropolitan Cleveland, Ohio. In 2006, 17.27% of poisoning related deaths were due to heroin-related intoxications. By 2011, 35.96% of poisoning deaths were heroin related. This statistic is reversed with regards to cocaine poisonings, 47.84% in 2006 versus 23.97% in 2011. Levels of polypharmacy have remained high over the years (present in over 50% of heroin deaths per year), while heroin-only deaths have doubled since 2006. Although there has been a statistically significant increase in usage by females, demographics show decedents positive for heroin typically are single, white, males between the ages of 20-30 and 50-60. During this period of time heroin related deaths increased by 77% in the city of Cleveland and 176% in the suburbs.

Conclusions: This study demonstrates that there has been an increase in heroin-related deaths in the City of Cleveland and the surrounding suburbs. These findings are consistent with other reports that heroin is a major cause of concern throughout the Midwest and Northeastern regions of the United States. A possible explanation for this increase could be the changing formulations of prescription Opiates, which make abuse more difficult. Another explanation could be the low cost and high availability of heroin relative to prescription Opiates.

Keywords: Heroin, Opiates, Medical Examiner, Demographics, Ohio

P54
Hair Analysis for Zolpidem by Liquid Chromatography / Tandem Mass Spectrometry

Cynthia L. Morris-Kukoski*, Eshwar Jagerdeo; Federal Bureau of Investigation Laboratory Chemistry Unit, Quantico, VA, USA

Background/Introduction: Zolpidem is an imidazopyridine sedative-hypnotic drug that has been implicated in forensic toxicological casework. Although zolpidem has a short plasma elimination half-life, it is easily detectable in overdose cases or in driving under the influence cases by either gas chromatography/mass spectrometry or liquid chromatography/mass spectrometry. In cases of suspected drug-facilitated sexual assault, underivatized zolpidem metabolite may be detected for approximately 24-36 hours but only by liquid chromatography/mass spectrometry. Hair analysis to detect exposure to zolpidem may aid in a drug-facilitated sexual assault investigation. Liquid chromatography/tandem mass spectrometry provides both the specificity and sensitivity required for this analysis.

Objective: The objective of this study was to validate a method for the analysis of hair samples for zolpidem.

Methods: Hair samples were decontaminated with a series of methylene chloride washes and dried before grinding. Samples were ground to a powder using a cryogrinder. Resulting powder was spiked with deuterated internal standard (d6-zolpidem); and incubated overnight with saturated monobasic ammonium phosphate buffer. Hair buffer solution was centrifuged and the liquid portion was extracted in methylene chloride:diethyl ether mixture (80:20). After gentle rotation and centrifugation, the organic layer was removed, taken to dryness using an evaporator and reconstituted in acetonitrile:water (1:1) for analysis. The final extracts were analyzed on an ABI 4000 Qtrap by Liquid chromatography / tandem mass spectrometry. The LC column used was a Waters Xbridge ® Phenyl 3.0 mm x 50 mm x 5 µm analytical column. Mobile phase consisted of 0.03% ammonium hydroxide in Optima Grade water and Acetonitrile. Chromatographic separation was achieved using a mobile phase gradient from 10 to 90% Acetonitrile with a flow rate of 0.3 mL/minute; over a 15 minute analysis time. Source temperature is 650 degrees Celsius with an ionspray voltage of 5000 volts and an entrance potential of 10. The following MRM transitions were monitored: 308.25 -> 235.2; 263.1; 92.1; and 219.1 for zolpidem and 314.29 -> 235.1; 263.4; 92.1; and 219.2 for d6-zolpidem.

Results: This method was fully validated according to laboratory requirements for qualitative identification with a limit of detection of 1 picogram per milligram of hair (using a 50 milligram hair sample). Testing via non-segmental analysis was positive for zolpidem in three out of four children that had allegedly been drugged and sexually molested for an undetermined time frame.

Conclusion/Discussion: This method was fully validated for qualitative analysis of zolpidem in hair samples. It has since been applied to several forensic cases with both positive and negative findings.

Keywords: Zolpidem, Hair Analysis

P55

Drug-Facilitated Sexual Assault Drug Metabolites - The Need for Production and Identification

Cynthia L. Morris-Kukoski*, Jason E. Schaff; FBI Laboratory Chemistry Unit, Quantico, VA, USA

Background/Introduction: The Society of Forensic Toxicologist Drug-Facilitated Sexual Assault Committee 2005 Common DFSA Drugs/Metabolites List contains approximately 59 parent drugs and 19 metabolites. Despite the fact that most drugs are excreted in the urine as metabolites, most laboratories continue to look for only parent drugs due to lack of commercially available metabolite drug reference standards.

Objective: The FBI Laboratory evaluated the availability of drug metabolites in the above mentioned DFSA list. Furthermore, newer drugs/drug metabolites recently detected in alleged DFSA cases but not on the DFSA list were evaluated for ease of purchase.

Methods: The SOFT DFSA 2005 Drug List, new sedative drugs released over the last five years, and the FBI Laboratory in-house library were reviewed. Several commercial drug reference standard companies inside and outside the continental United States were contacted. All reasonably priced drugs were obtained. Standards were analyzed via gas chromatography/mass spectrometry and/or liquid chromatography/high resolution tandem mass spectrometry for incorporation into our in-house drug library. Over 170 layouts were created for liquid chromatography-high resolution mass spectrometry drug monitoring for alkaline drugs and benzodiazepines.

Results: None of the three antihistamine drug metabolites listed on the SOFT DFSA 2005 List are readily commercially available. Nineteen drug metabolites are available that could be added to the next revision of the SOFT DFSA List. A review of FBI Laboratory case files looking for drugs found in DFSA cases that were not found on the DFSA list include: trazodone; hydroxyzine / norchlorcyclizine / cetirizine; promethazine; zolpidem metabolite; midazolam/hydroxyl-midazolam; venlafaxine / n / o-desmethylvenlafaxine; tramadol /desmethyltramadol. Inclusion of recently released sedating drugs and their metabolites identified an additional six drugs and five metabolites.

Conclusion/Discussion: In order to lengthen detection times of DFSA drugs, commercially available drug metabolite should be obtained and samples should be hydrolyzed accordingly. Several new pharmaceutical agents with sedating properties are available and should also be considered as useful additions to the SOFT DFSA 2005 List.

Keywords: Drug-Facilitated Sexual Assault, Drug Metabolites

P56

Automated Extraction and Analysis of Pain Management Drugs Using Disposable Pipette Extraction and LC/MS/MS

Oscar G. Cabrices*[1], Fred D. Foster[1], John R. Stuff[1], Edward A. Pfannkoch[1], William E. Brewer[2]; [1]GERSTEL, Inc., Linthicum, MD, USA, [2]University of South Carolina, Columbia, SC, USA

Background/Introduction: Solid phase extraction (SPE) is a widely used; proven method for sample preparation and sample clean-up in the field of forensic analysis. Most SPE products require relatively large volumes of solvent leading to increased time for sample processing, increased cost per sample and higher limits of detection. Disposable Pipette Extraction (DPX) was developed as an alternative to traditional SPE; combining efficient and rapid extraction with significantly reduced solvent consumption. DPX is a novel dispersive solid-phase extraction device that uses sorbent loosely contained in a pipette tip to efficiently mix with sample solutions. The main advantages of DPX technology are: rapid extractions; high recoveries; negligible solvent waste is generated; and the extractions can be fully automated and coupled to chromatographic injections.

Objective/Methods: This study focuses on the automated extraction of small sample volumes coupled to LC/MS/MS in order to provide high throughput analysis of common pain management drugs. Using a GERSTEL MPS 2 autosampler, DPX extractions of hydrolyzed urine were performed; using a reversed phase (DPX-RP-S) sorbent. The resulting eluents from the automated DPX extractions were then introduced into an Agilent 6460 LC/MS/MS instrument. Coupling DPX to LC/MS/MS provides rapid, just-in-time sample preparation for high throughput analysis. The DPX extraction removes potential matrix interferences and ion suppression; high sensitivity is therefore achieved.

Several important pain management drugs have been quantified in biological fluids by automated DPX followed by derivatization and GC/MS. However this approach was limited to compounds amenable to derivatization such as nordiazepam and α-OH-alprazolam. DPX/LC/MS/MS was chosen to eliminate the need for derivatization and address a broader range of pain management drugs.

Results: The automated DPX cleanup method using the dual head GERSTEL MPS 2XL robotic sampler for pain management drug screenings in urine was developed to provide cycle times of approximately 7 min/sample allowing throughput of over 200 samples per day. A comprehensive panel of 49 pain management drugs (opiates, opioids, benzodiazepines, antidepressants, muscle relaxants, anticonvulsants, hallucinogens and stimulants) can be rapidly and reproducibly extracted from hydrolyzed urine samples using an automated DPX cleanup procedure coupled to an Agilent 6460 LC/MS/MS system. Extraction efficiencies for all drug classes are shown to be greater than 70% with %RSDs less than 10%, in most cases. Linear calibration curves resulting in R^2 values 0.99 or greater were acheived with LOQs lower than the minimum reportable limits for the majority of pain management drugs analyzed. The DPX-LC/MS/MS method provided good accuracy and precision averaging 98.0% (range: 77% - 108%) accuracy with 4.2 % RSD (range: 1.0% - 12.8%) for all analytes.

Conclusion/Discussion: Data show the use of an Agilent 6460 LC/MS/MS instrument to be a highly sensitive procedure for the initial screening of pain management drugs, allowing their respective limits of detection to be met and obtaining good linearity for calibration curves. Coupling DPX to LC/MS/MS provides high throughput and minimizes matrix interferences.

Keywords: Solid Phase Extraction, Lab Automation, LC/MS/MS

P57

Nasal Swab: Correlation with Postmortem Blood, Vitreous Humor and Urine in Cocaine Results

Emilirma Vázquez-Reyes*, Kazandra Ruiz-Colón, Maruiz Marrero-Ilarraza, Margarita Meléndez-Negrón, Irma Rivera-Diez, María S. Conte-Miller; Institute of Forensic Sciences of Puerto Rico

Introduction: A rapid review of forensic literature show many studies of cocaine analysis in blood, vitreous humor and urine. But there are almost no references about the use of nasal swab in postmortem cases. At the Toxicology Laboratory in the Institute of Forensic Science of Puerto Rico (IFSPR) all cases are routinely analyzed by Enzyme-Linked ImmunoSorbent Assay (ELISA) and presumptive positives samples are confirmed by Gas Chromatography Mass Spectrometry (GC/MS).

Objective: The aim of this study is to correlate the nasal swab analysis with a variety of biological samples (blood, vitreous humor and urine) and the overall output of the cocaine assay at the IFSPR.

Method: A retrospective study was undertaken by evaluation of cocaine assay results for the years 2009, 2010 and 2011. Data for each matrix was evaluated to determine the correlations and trends between blood, vitreous humor, urine and nasal swab for the analysis results.

Results: Over the three year period, a total of 8,375 cases were submitted for drug analysis and screened for the presence of cocaine. Only 50.3% of those cases (4,216) contained all four matrices (blood, vitreous humor, urine and nasal swab). Just 556 out of 4,216 (13.1%) cases gave positive results in the four matrices. From 919 presumptively positive bloods, 861 gave a positive result by GC/MS. The other fifty-eight (58) were confirmed by GC/MS in other matrices such as vitreous humor and urine. No confirmation was made in nasal swab. Evaluation of data from the 861 confirmed positive bloods gave positive results also in vitreous humor (95.6%), urine (99.0%) and nasal swab (64.2%). In cases that did not have all four matrices, few nasal swabs were ELISA positive and not all confirmed on GC/MS.

Conclusion: The strong correlation between the blood, vitreous humor and urine demonstrates that these matrices are still being an important medium for toxicology testing when attempting to make a determination of cocaine intoxication. Data suggests that ELISA positive or negative results for blood, vitreous humor and urine not necessary give positive or negative results in nasal swab, probably because the different routes of administration or due to involuntary exposure. Therefore, nasal swab confirmations give useful information only if a biological matrix also gives positive to cocaine/benzoylecgonine.

Keywords: Correlation, Nasal Swab, Cocaine, GC/MS

P58 Detection of Morphine in Oral Fluid vs. Whole Blood in Chronic Pain Patients

Andrew Mummert*[1], Andrea R. Terrell[1], Christine Moore[2]; [1]AIT Laboratories, Indianapolis, IN, USA, [2]Immunalysis Corporation, Pomona, CA, USA

Background: Pain medication compliance monitoring is a very important tool for physicians to ensure compliant use of prescribed medications; and identify potential situations where patients may be diverting, substituting, or abusing their prescribed pain medications. Although urine and blood currently are the most common biological samples, oral fluid can potentially be used to monitor a chronic pain patient's compliance with their prescription regimen. Oral fluid collection is minimally disruptive, and as a result, patients, sample collectors and physician offices benefit from the ease and speed of collection as compared to drawing blood or collecting urine.

Methods: Blood and oral fluid was collected from 107 chronic pain patients over a 3-month period at three different pain management practices. The blood was sent to AIT Laboratories for routine analysis, which included screening on a Tecan Genesis 200/8 using Immunalysis ELISA kits, followed by confirmation and quantitation of presumptively positive results by LC/MS/MS. The oral fluid was sent to Immunalysis and was screened on a Tecan Genesis and then confirmed by LC/MS/MS.

Results: 29 of the 107 patients screened and confirmed positive for morphine in blood and 25 of the 107 patients screened and confirmed positive for morphine in oral fluid. 72% of the patients had an oral fluid to blood ratio of less than 2.5. In this group; we saw a correlation coefficient of 0.72. The remaining patients who had an oral fluid to blood ratio greater than 2.5 were further investigated. The high concentrations of morphine in oral fluid compared to blood could be attributed to the prescribed dose, the frequency of dosing, inter-individual differences in absorption and metabolism, and/or the time interval between last dose and specimen collection.

Conclusion: A high proportion of patients receiving chronic opioid therapy showed good correlation between oral fluid and blood concentrations of morphine. These results provide promising evidence that oral fluid could be a good alternative or adjunct specimen to blood or urine.

Keywords: Oral Fluid, Pain Compliance Monitoring, Oral Fluid and Blood Comparison, ELISA

P59

Ten Second Sample to Sample Analysis of Drugs in Oral Fluid by Laser Diode Thermal Desorption (LDTD) Combine to Mass Spectra Technology

Ted Palashis[1], Dean Fritch[2], **Serge Auger*[3]**, Alex Birsan[3], Pierre Picard[3]; [1]Overbrook Scientific, Inc, Boston, MA, USA, [2]OraSure Technologies, Bethlehem, PA, USA, [3]Phytronix Technologies Inc, Quebec, P.Que, Canada

Objectives: Oral fluid analysis is an increasingly useful and non-invasive method that has facilitated laboratory analysis for many drugs of abuse. Using the Intercept device in combination with the Laser Diode Thermal Desorption (LDTD) technology, we propose to develop an ultra-fast and accurate method for drug analysis. This technique is applied to quantify the following drugs: Amphetamine; Methamphetamine; MDEA; MDA; MDMA; Methadone; PCP; Cocaine; THC; Fentanyl; Norfentanyl; Secobarbital; Phenobarbital and Butalbital.

Materials and Methods: A calibration curve and quality control material are spiked in the Intercept extraction buffer. A Liquid-Liquid Extraction (LLE) procedure is performed to isolate the drug before adding extracted sample in a specially constructed 96-well plate. For basic drugs (Amphetamine; Methamphetamine; MDEA; MDA; MDMA; Methadone; PCP; Cocaine; Fentanyl and Norfentanyl), a LLE in basic pH is used: 100 µL of sample; 20 µL IS; 100 µL NaOH (0.1N in water) and 600 µL Ethyl Acetate. For acidic drugs (Secobarbital; Phenobarbital and Butalbital, a LLE in acid pH is used: 100 µL of sample; 20 µL IS; 100 µL HCl (0.1N in water) and 600 µL 1-Chlorobutane. For the neutral drug (THC), the following procedure is used: 100 µL sample; 20 µL IS; and 300 µL 1-Chlorobutane. For all extractions, the organic phase is transferred into another tube, evaporated to dryness and reconstituted with a mixture of Methanol:Water (75/25), then 5 µL is transferred to a 96-well plate for analysis.

Results: The calibration curves show excellent linearity over the range with $r^2 > 0.99$ for all molecules. The following range of quantification were established on the diluted oral fluid sample: 10 to 1000 ng/ml for Amphetamine (LOD: 1ng/ml), Methamphetamine (LOD: 1ng/ml), MDEA (LOD: 1ng/ml), MDA (LOD: 1ng/ml) and MDMA (LOD: 01ng/ml); 5 to 500 ng/ml for Methadone (LOD: 1ng/ml); 1 to 100 ng/ml for PCP(LOD: 0.5ng/ml) and THC (LOD: 0.5ng/ml); 2 to 200 ng/ml for Cocaine (LOD: 0.5ng/ml); 0.5 to 50 ng/ml Fentanyl (LOD: 0.1ng/ml) and Norfentanyl (LOD: 0.1ng/ml); 20 to 2000 ng/ml for Secobarbital (LOD: 2ng/ml), Phenobarbital (LOD: 2ng/ml) and Butalbital (LOD: 2ng/ml). The inter-day and intra-day imprecision was evaluated to be less than 6.8 % for all analytes. The wet stability was evaluated to three days and the Lazwell plate (dry sample) stability was also determined to be three days.

Conclusions: LDTD technology provides unique advantages in developing an ultra-rapid analysis method for drugs of abuse in oral fluids. The collection technique using the Intercept device and subsequent analysis method has demonstrated accurate, precise and stable results for a wide range of drugs.

Keywords: High Throughput, LDTD-MS/MS, Oral Fluids, Drug of Abuse

P60
Noroxycodone Concentration in Oral Fluid: A Retrospective Study

Margaux Garnier, **Cynthia Coulter*,** Christine Moore; Immunalysis Corporation, Pomona, CA, USA

Introduction: Detection of metabolites in oral fluid can be helpful for medical monitoring clinics that need to determine if the drug is in fact being ingested and is metabolized by the body. Oxycodone confirmation data from oral fluid samples received into our laboratory were reexamined. Previously confirmed samples were re-extracted and analyzed for oxycodone and its metabolites noroxycodone and oxymorphone.

Objective: To analyze previously confirmed oxycodone positive samples in order to determine a lower limit concentration range for its metabolites noroxycodone and oxymorphone.

Methods: Oral fluid samples were retrospectively analyzed using LC-MS/MS for oxycodone and its metabolites noroxycodone and oxymorphone. Specimens which confirmed above 10ng/ml for oxycodone, noroxycodone, and oxymorphone were noted.

Results: Of 31 specimens analyzed, 18 specimens confirmed for both oxycodone and noroxycodone. None of the samples confirmed for oxymorphone above 10ng/ml. The percentages of noroxycodone to oxycodone in those samples ranged from 4.9% to 50%. This wide range and those samples which did not confirm positive for oxycodone, noroxycodone or oxymorphone may be accounted for by the variability in dosage, the time of dosage and oral fluid collection, and the period of time the samples were stored since this was not a controlled study. The average percentage of the 18 specimens is 17.6% of noroxycodone.

Conclusion: Noroxycodone is a major metabolite of oxycodone in oral fluid. Monitoring noroxycodone would validate that the patient in question is in fact taking the drug. A concentration for noroxycodone of 15% of the oxycodone cut-off is suggested. Future studies will focus on fresh samples analyzed in real time in order to better monitor oxycodone metabolites.

Keywords: Oral Fluid, Oxycodone, Noroxycodone

P61

Identification of THC-COOH in Oral Fluid Using LC-MS/MS: Essential Analyte in Workplace Testing

Cynthia Coulter*, Margaux Garnier, Christine Moore; Immunalysis Corporation, Pomona, CA, USA

Objective: The detection of the marijuana metabolite THC-COOH in oral fluid (OF) minimizes the possibility of identifying individuals from testing positively for marijuana after passive exposure to smoke. Its determination requires instrumentation which is extremely sensitive since the concentrations present are very low compared to the parent drug, THC. Our objective was to develop a procedure for analysis using LC-MS/MS and apply the method to authentic specimens.

Methods: To achieve the requisite sensitivity for THC-COOH an Agilent Technologies 6490 LC-MS/MS instrument, base hydrolysis and extraction of the drug from OF, and derivatization were employed. Samples were hydrolyzed and extracted according to a fully validated and previously published method. Once samples were eluted and evaporated to dryness TPP(10mM); DPDS(10mM) and 2-PA(10µg) were added to the vial capped and heated for 15 min at 60°C. The derivative was allowed to cool and evaporated to dryness then reconstituted in ACN: deionized water (50:50; 25µL). Extracts were run in (+) ESI with Jet Stream technology with a 20µL injection on a Stable Bond C-18 (2.1x50mm x 1.8µm) column. Mobile phase A consisted of ammonium formate (pH 6.4) with B as 0.5% formic acid in ACN. The pump program held B at 70% for 2 minutes; by 4 minutes B was held at 90% with a stop time of 7 minutes. The flow rate was held constant at 0.2 mL/min. All voltages, gas flows, and temperatures were optimized for the highest level of sensitivity and reproducibility. Transitions for THC-COOH and THC were **435.3>327.0**; 435.3-299.0 and **315.3>193.3**; 315.3-123.3 respectively. Qualifying transitions for THC-COOH 2-PA were 41.6%-62.4% and THC were 47%-71%.

Results: The procedure was developed and fully validated. The limit of quantitation for THC-COOH was 10pg/mL and linearity was established up to 1000pg/mL. Six replicate analyses over 5 days (n=30) at 12pg/mL and 75pg/ml resulted in an impression of 6.5% and 1.9% for THC-COOH and replicates at 12ng/ml and 75ng/ml resulted in an impression of 4.0% and 4.8% for THC. Authentic oral fluid specimens as well as proficiency samples were analyzed for both THC and THC-COOH. In the case of two authentic oral fluid specimens, THC levels were at 1.2 and 2.1ng/mL with corresponding THC-COOH levels at 14 and 23pg/mL; illustrating the need for very sensitive methods. Other samples ranged in THC concentrations from 11 - 3440ng/mL with corresponding THC-COOH levels 34 - 1651pg/mL. There appeared to be a good correlation between the THC and THC-COOH concentrations in authentic oral fluid samples. All proficiency samples reported for THC and THC-COOH were within 10% of the target value.

Conclusion: An analytical procedure for the simultaneous determination of THC and THC-COOH in oral fluid with the required sensitivity needed to analyze THC-COOH in oral fluid has been developed using LC-MS/MS instrumentation. The adapted use of the 2-picolylamine (2-PA) derivative increased the sensitivity of THC-COOH by 20-fold and had no impairing effects on THC. The method when applied to authentic specimens provided good correlation between the two compounds THC and THC-COOH.

Keywords: THC-COOH, Oral Fluid, LC-MS/MS

P62
Confirmation of Multiple Drugs in Oral Fluid Using LC-MS/MS

Cynthia Coulter*, Margaux Garnier, James Tuyay, Christine Moore; Immunalysis Corporation, Pomona, CA, USA

Introduction and Objective: In 2011, the Drug Testing Advisory Board of the Substance Abuse and Mental Health Service Administration (SAMHSA) recommended that oral fluid be allowed as a specimen in federal workplace drug-testing programs. The board had previously considered oral fluid, but had determined it was not an allowable matrix due to disadvantages such as inadequate or unknown volume for analysis; drug absorption to collection pads; and low drug concentrations. The new recommendation is recognition that technology has largely overcome these problems. Further, it was recommended that the synthetic opioids oxycodone and hydrocodone be added to the test panel. The objective was to develop and validate a simple rapid method to allow the simultaneous analysis of amphetamines, PCP, opioids, and cocaine and its metabolite. The method provides reliable quantitative results with the use of appropriate internal standards while limiting the amount of sample utilized for analysis, a distinct advantage in oral fluid testing. A single solid-phase extraction was performed to encompass all the drugs in the SAMHSA panel, except THC, and the extracts were analyzed on an Agilent 6430 LC-MS/MS.

Methods: Oral fluid samples collected with the Quantisal™ collection device are routinely received in our laboratory. Samples are initially screened by immunoassay with positives carried on to confirmation. A calibration range of 1 - 200ng/mL was used and satisfies the SAMHSA federally proposed cut-offs. Appropriate deuterated internal standards were added to 1mL of sample (750µl buffer + 250µl oral fluid) and buffered to pH 6.0. Clin II (SPEWare) solid-phase columns were conditioned with methanol (2mL) and 0.1M phosphate buffer (pH 6.0; 2mL). Samples were loaded and allowed to flow through to dryness; then washed with deionized water (2mL), 0.1M HCl (2mL), methanol (1mL) and ethyl acetate (1mL). Columns were dried under nitrogen for 5 minutes. Analytes eluted with methylene chloride: methanol: ammonium hydroxide (78:20:2 v/v; 2mL). Eluent is dried and reconstituted in mobile phase: 20mM ammonium formate (pH 3.6); methanol (70:30; 50 µl) for analysis. The mobile phase consisted of 85% ammonium formate pH 6.4 (A) and 15% methanol (B). At 6 min B is at 80% with a constant flow of 0.7mL/min. Stop time is 6min with a 3min equilibration period. Two transitions were monitored for each analyte and all transitions were optimized for their appropriate fragmentation voltage and collision energies.

Results: The method was optimized using calibrators which covered the concentration range proposed by SAMHSA using the minimum volume of oral fluid for multiple drugs with one extraction and one injection. The procedure was validated according to standard protocols and applied to specimens received into our laboratory.

Conclusion: A simple extraction and analysis procedure has been developed for the SAMHSA proposed drugs of abuse in oral fluid with the exception of THC. Consolidation of methods can circumvent the limitations of sample volume, and lower laboratory costs, as well as extending the life of the instruments and columns.

Keywords: Drugs of Abuse, Oral Fluid, LC-MS/MS

P63
Detection of Benzoylecgonine in Meconium Using Immunalysis ELISA Kits

Joel Bailey*, Andrew Mummert, Andrea Terrell; AIT Laboratories, Indianapolis, IN, USA

Background: Meconium is a black, tar-like substance that comprises the first stools from newborns and it can tell us about prenatal exposure to various drugs of abuse. Analyzing meconium for drugs of abuse is unique and not done in many laboratories.

Methods: The previous method used to screen meconiums was using Immunalysis ELISA kit for Cocaine/BE on a Dynex DSX analyzer. It was found that the absorbance separation between the quality controls and the cutoff calibrator was not enough at the lower portion of the curve. This resulted in many negative meconium samples to have an absorbance close to the absorbance of the cutoff calibrator of 10 ng/mL. Since this testing is very sensitive in nature; these samples close to the cutoff were forced to confirm. Every sample forced to confirm was negative; therefore wasting time and money running samples that didn't need to be tested. m-Hydroxybenzoylecgonine is the predominant metabolite in meconium. The Cocaine ELISA plate has very little cross reactivity with this method while the BE only ELISA plate cross reacts well with m-Hydroxybenzoylecgonine. The method was switched to the BE only plate.

Results: Within-run precision and between run precision of 96 replicates per standard (negative control, low positive control, cutoff, and high positive control) based on a 10 ng/mL cutoff resulted in coefficient of variations to be less than 15% over a 4-day period (24 replicates per standard on each day). Standards of 0, 5, 10, and 20 ng/mL all achieved an absorbance separation of at least two standard deviations in-between them on all four days. Method comparison studies showed that there is no need to force samples to confirm any longer due to the improved sensitivity of the Immunalysis Cocaine Metabolite kit at the lower end of the calibration curve.

Conclusion: Switching to the Immunalysis Cocaine Metabolite kit created much more absorbance separation in between the low quality control, the cutoff calibrator, and the high quality control therefore effectively eliminating false positives.

Keywords: ELISA, Meconium, Cocaine, Screen

P64

Fast, Robust LC-MS/MS Method for Quantification of Multiple Drug Classes for Therapeutic Drug Monitoring

Amanda Rigdon*, Chris Denicola, Carrie Sprout, Sharon Lupo, Ty Kahler; Restek, Bellefonte, PA, USA

Introduction: As demand for therapeutic drug monitoring rises, laboratories are under increased stress to implement streamlined, cost-effective testing procedures. As with any high-volume application, methods developed for therapeutic drug monitoring must be fast, robust, easy to implement, and minimize costs associated with laboratory consumables and technician time. The method presented in this poster is suitable for the quantification of 29 drug compounds in urine across four drug classes in a total run time of 5.5 minutes.

Objective: The objective of this work was to develop a fast, robust LC-MS/MS method for the quantification of 29 compounds in urine from several classes including opiates, benzodiazepines, tricyclic antidepressants, anticonvulsants, and antihistamines using a cost-effective HPLC column.

Methods: The method discussed in this poster employed a Shimadzu UFLCXR HPLC coupled to an AB SCIEX API 4000 MS/MS and a 5µm Ultra Biphenyl 100mm x 2.1mm analytical column. A matching guard column was also used. The mobile phases were 0.1% formic acid in water and 0.1% formic acid in acetonitrile. The gradient profile began at 10% B at 0.0 minutes, held at 10% B for 1 minute, and ramped to 100%B in 2.5 minutes. The mobile phase was held at 100% B for 0.5 minutes, and the column was re-equilibrated for 1.5 minutes. The MS/MS instrument employed positive ESI and was operated in MRM mode, with three MRMs monitored for each analyte and two MRMs monitored for each internal standard. Prior to analysis, urine samples were enzymatically hydrolyzed, then diluted 10x in 10% B mobile phase containing 4ng/mL internal standards (codeine-d3, doxepin-d3, and diazepam-d5).

Results: LOQs for all compounds ranged from 1 - 25ng/mL, with signal-to-noise ratios for all quantifier ions ≥ 10 and all qualifier ions ≥ 3. Linearity for all compounds was greater than $r = 0.9911$, with r values for 25 compounds ≥ 0.9990. Accuracy at LOQ ranged from 88% - 113%, and %CV at LOQ ranged from 1% - 23%, with 23 of 29 compounds falling within 90% - 110% accuracy and %CV.

Conclusion/Discussion: Based on the data presented here, this method, accompanied by a fast sample preparation option, is suitable for the quantification of 29 drug compounds and metabolites in urine for the purposes of therapeutic drug monitoring.

Keywords: Therapeutic Drug Monitoring, LC-MS/MS, HPLC

P65

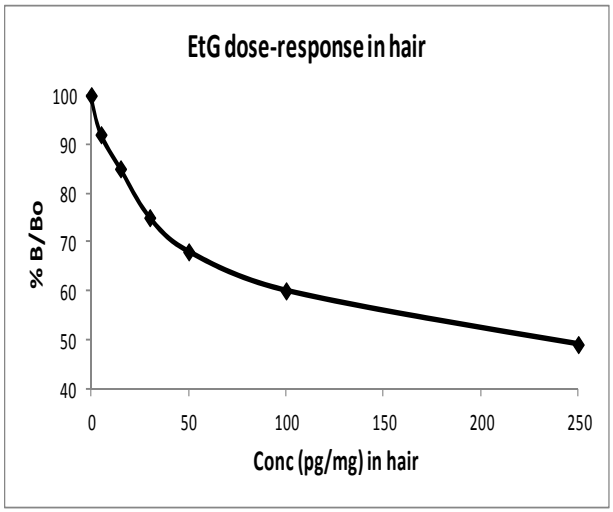
A Novel ELISA for Detection of the Alcohol Biomarker Ethyl Glucuronide in Hair

Warren C. Rodrigues*, Philip Catbagan, Catherine Castro, Kerry Wang, Guohong Wang, Christine Moore; Immunalysis Corporation, Pomona, CA, USA

Background: Alcohol addiction is a worldwide problem affecting millions of people. In cases of driver license reinstatement, monitoring sobriety and/or abstinence becomes significant. Ethanol can be measured by means of various biomarkers in the body and by direct metabolites such as ethyl glucuronide (EtG). Following alcohol consumption, most of the ethanol is converted to acetaldehyde in the liver, but a small amount is converted to EtG. The window for detection of EtG is up to 80 hours in urine and 18 hours in blood. In order to monitor sobriety over longer periods of time, it becomes necessary to utilize hair testing, since EtG remains in the hair follicles for up to weeks and months and can be a marker of chronic heavy drinking. This study reports the first immunoassay method, developed to meet the screening cutoff of 30 pg/mg suggested by the Society of Hair Testing (SoHT).

Method: Hair specimens (20 mg) were weighed and transferred to test tubes; 1 mL of a proprietary hair extraction buffer was added and sonicated at 60°C for 2 hours. Specimens (125 µL) were pipetted directly onto microtiter plate in duplicate and incubated for 30 min, followed by enzyme conjugate (60 min incubation). The plates were washed, then incubated with substrate (30 min). The reaction was then stopped with 1N HCl and read at 450 nm using a microplate reader.

Results and Validation: The screening cutoff of the assay is 30 pg/mg in hair. The dose-response curve is shown below.



Specimen #	*ELISA (P/N)*	*LC-MS (pg/mg)*	*Hair color*
1	N	20 - 50	Dark brown
2	N	4 - 20	Gray
3	N	> 100	Red
4	P	Neg	Blonde
5	N	4 - 20	Gray
6	P	> 100	Dark brown
7	N	Neg	Brown
8	N	20 - 50	Black
9	N	4 - 20	Red
10	P	>100	Black
11	N	Neg	Black
12	N	4 - 20	Black
13	N	4 - 20	Brown
14	N	20 - 50	Black
15	N	Neg	Black
16	N	4 - 20	Black
17	P	>100	Black
18	N	4 - 20	Brown

The intra and inter-day precisions were found to be <10%. The assay is highly specific for EtG only and shows no cross-reactivity with ethyl sulfate (EtS), ethanol, other common alcohols or other glucuronides. The assay was further validated with 18 hair specimens obtained from a clinical laboratory. One false negative was observed at the assay cutoff of 30 pg/mg, when compared with the LC-MS confirmation results. This was attributed to the limited hair specimen available for the ELISA screen, which perhaps skewed the result.

Conclusion: The described method is a preliminary, but precise, screening assay specific for detection of ethyl glucuronide in hair and meets SoHT testing guidelines for a 30 pg/mg cutoff.

Keywords: Ethyl Glucuronide, EtG, Hair ELISA

P66

Automated Extraction and Analysis of Drugs of Abuse in Oral Fluid Using Disposable Pipette Extraction (DPX) and LC/MS/MS

William E. Brewer*[1], Oscar G. Cabrices[2], Fred D. Foster[2], Edward A. Pfannkoch[2]; [1]DPX Labs, Columbia, SC, USA, [2]GERSTEL, Inc, Linthicum, MD, USA

Introduction: Oral fluid has become increasingly used as a specimen in many areas of forensic and clinical interest. The biggest challenges associated with oral fluid analysis are the requirement of low detection limits and complex sample matrix interferences. Due to these challenges, sample preparation is required prior to analysis. The solid-phase extraction method developed in this study used disposable pipette extraction (DPX) for comprehensive analysis of drugs of abuse using LC/MS/MS.

Objective: To develop an automated, rapid and comprehensive method of extracting and analyzing drugs of abuse in oral fluid using LC/MS/MS.

Methods: All drug standards were purchased from Cerilliant Corporation (Round Rock, TX, USA). The drugs and their corresponding deuterated internal standards include 1) opiates: morphine, oxymorphone, hydromorphone, codeine, oxycodone, hydrocodone); 2) stimulants: cocaine, benzoylecgonine, cocaethylene, amphetamine, methamphetamine, MDMA, MDEA and MDA; and 3) hallucinogens: PCP and THC.

Automated DPX extractions were performed using an automated prep sequence which included the steps of:

1. aspirating 500 µL stabilized oral fluid solution into a DPX-WAX tip (DPX Labs, Columbia, SC)
2. mixing with the sorbent and dispensing to waste
3. washing with water
4. eluting with 250 µL methanol.

The extracts were either first diluted by adding 750 µL water and injected directly, or solvent evaporated using the GERSTEL mVAP station, and then reconstituted in 250 µL 20% methanol in water.
All analyses were performed using a GERSTEL MPS 2XL autosampler configured with an active washstation coupled to an Agilent 6460 LC/MS/MS instrument with a Poroshell EC-C18 column (3.0 x 50mm, 2.7 µm). Sample injections were made using a 6 port (0.25mm) Cheminert C2V injection valve.
The LC mobile phase used was: A - 5mM ammonium formate with 0.05% formic acid; B – 0.05% formic acid in methanol. The gradient started at 95% A, then ramped to 95% B and held there for a total run time of 6.5 min. The flow rate was 500 µL/min.

Results and Discussion: The automated DPX drug extraction and analysis method provided extraction efficiencies greater than 70% for all drugs screened with RSDs less than 15%. In addition, good linearity was achieved (R^2 values of 0.98 or greater) for all drugs tested. Detection limits were found to be approximately in the range of 0.5 to 10 ng/mL for all drugs using an automated solvent evaporation station, and in the 2.0 to 40 ng/mL range without the concentration step. Without concentration, the sample preparation takes approximately five min to perform. Therefore, DPX extractions are "just-in-time" with fast LC methods. However, an additional 5 min per sample is required with solvent evaporation. Nevertheless, "just-in-time" sample preparation was still obtained using a ten min LC/MS/MS run (due to the analysis of THC, which elutes relatively late).
The biggest advantage of this DPX method is that one method could be used to simultaneously extract all of the drugs of interest with high recoveries with very little ion suppression. Only a few opiates had indications of ion suppression effects.

Keywords: Dispersive Pipette Extraction, Oral Fluid, LC/MS/MS

P67

High Throughput Method for Quantification of 11-nor-9-Carboxy-THC in Urine by Laser Diode Thermal Desorption (LDTD) Combine with Mass Spectra

Pierre Picard*[1], William P. Gibbs[2], Alex Birsan[1], Andrew J. Fischinger[2], Serge Auger[1];
[1]Phytronix Technologies Inc, Quebec, Canada, [2]Norchem Drug Testing, Flagstaff, AZ, USA

Objectives: 11-Nor-9-Carboxy-THC is a major metabolite of THC in urine. Detection and quantification of 11-Nor-9-Carboxy-THC in urine is traditionally performed by LC-MS/MS or GC/MS analysis. The Laser Diode Thermal Desorption (LDTD) technology combine with a Mass Spectra system (LDTD-MS/MS) allows complete analysis in ten seconds sample to samples. We propose to validate a quantitation method for 11-Nor-9-Carboxy-THC in urine at a concentration ranging from 3.5 to 600 ng/mL and compare with LC-MS/MS results.

Materials and methods: A calibration curve and quality control material are prepared in blank urine. Calibration curve, QC sample and patient specimens are spiked with Internal Standard containing 11-Nor-9-Carboxy-THC-d9. A liquid-liquid extraction is performed in acid pH: 100 µL sample; 20 µL internal standard; 100 µL HCl (0.1N in water) and 600 µL Hexane:Ethyl acetate:Isoamylalcohol (75:25:1). Upper phase is transferred, evaporated to dryness and reconstituted with a mixture of MeOH:Water (75/25) containing EDTA. Two microliters (2µl) are deposited directly into the 96-well plate made for LDTD-MS/MS analysis.

Results: The calibration curves show excellent linearity with r2 = 0.9991 and the limits of detection and quantification were established to be 3.5 ng/ml for the analytes while the upper limit was set at 600 ng/ml. This method is cross validated with the gold standard, LC-MS/MS, with 12 patient specimens. A good correlation between LC-MS/MS and LDTD-MS/MS is obtained with r2 = 0.9998 (with 40 authentic urine specimens). All negative samples correlated accordingly. The intra-day and inter-day imprecision was evaluated to be less than 10 %. The wet stability was evaluated to within 3-days and the Lazwell plate (dry sample) stability to be 3-days. Finally, no carryover is observed.

Conclusions: LDTD technology provides unique advantages in developing an ultra-fast method for analysis of 11-Nor-9-Carboxy-THC in urine. This method has demonstrated accurate, precise and stable results with a run time of 10 seconds.

Keywords: High Throughput, LDTD-MS/MS, THCC

P68

Continued Propoxyphene Use in the Pain Population Following Removal of the Medication from the U.S. Market

Robert West*, Amadeo Pesce, Charles Mikel, Perla Almazan, Sergey Latyshev, Cameron West, Elizabeth Gonzales; Millennium Research Institute, San Diego, CA, USA

Introduction: Propoxyphene (PPX) was a commonly prescribed opioid analgesic used to treat mild-to-moderate pain. On November 19, 2010, it was withdrawn from the U.S. market after studies showed that the drug was associated with serious or fatal cardiac arrhythmias. As with any medication removed from the market, people may continue to take the medication with or without a prior prescription. Urine drug testing is one method that can identify continued PPX use.

Objective: To observe the incidence and overall decrease of continued use of PPX following its withdrawal from the market in order to determine if continued testing of PPX is clinically relevant to physicians.

Methods: Between August 2008 and December 2011, 838,324 urine specimens from patients being treated for pain were analyzed using LC-MS/MS at Millennium Laboratories. Briefly, samples were prepared for analysis by the addition of internal standard and buffered glucuronidase. The mixture was incubated, filtered and injected into the LC-MS/MS instrumentation. Analysis was performed using an Agilent 1200 series binary pump paired with an Agilent 6410 QQQ mass spectrometer using multiple reaction monitoring. Chromatographic separation was performed using an acetonitrile formic acid water gradient running at 0.4 mL/min and a Zorbax SB C18 column (2.1 × 50 mm, 1.8 μm). The lower limits of quantitation and upper limits of linearity for both PPX and norpropoxyphene (NPPX) were 100 and 100,000 ng/mL respectively. A retrospective study was conducted using these de-identified specimens to determine the presence of propoxyphene. Results were compared from two periods: 1) prior to PPX removal from the market, 2) after PPX removal from the market.

Results: From August 2008 to November 2010, prior to removal from the market, the percentage of patients taking PPX decreased from 11% to 4% (PPX median concentration 581 ng/mL, NPPX median concentration 3,306 ng/mL). From December 2010 to December 2011, despite removal from the market, patients continued to take PPX; and, the percentage of specimens positive for the presence of PPX decreased from 2.7% to 0.45% (PPX median concentration 384 ng/mL; NPPX median concentration 1,554 ng/mL). Prior to removal from the market, the range of values for both PPX and NPPX were 100 to > 100,000 ng/mL. After removal from the market, the range of values for PPX was 100 to > 100,000 ng/mL and 100 to 83,649 ng/mL for NPPX.

Conclusions: A small proportion of patients being treated for pain continue to utilize propoxyphene, despite removal from the U.S. market. However, the results of this retrospective data analysis demonstrate that overall use of propoxyphene by the pain population has continued to decline since its removal.

Keywords: Propoxyphene, Urine Drug Testing, LC-MS/MS, Withdrawal of Drug, Patients with Pain

P69

Driving Under the Influence of Drugs (DUID) Cases Involving New Synthetic Stimulants in New Mexico

Ginger L. Baker*, Protiti Sarker, Rong-Jen Hwang; Toxicology Bureau, Scientific Laboratory Division, New Mexico Department of Health, Albuquerque, NM, USA

Introduction: New amphetamine-related designer drugs, including cathinone and piperazine derivatives, have emerged on the recreational drug market. These formulations have wide-spread appeal for young people, and in the United States they have been sold as "bath salts", "plant food" and "research chemicals" by smoke shops and over the Internet as legal alternatives to controlled stimulants. In September 2011, the US Drug Enforcement Agency issued an "emergency scheduling authority" to control the synthetic cathinones: mephedrone (4-methylmethcathinone; 4-MMC), methylone (3,4-methylenedioxymethcathinone, MDMC) and 3,4-methylenedioxypyrovalerone (MDPV).

Objective: Pharmacological activity of the synthetic stimulant derivatives involves inhibition of the reuptake of brain neurotransmitters dopamine and norepinephrine. CNS and psychoactive effects of these stimulant drugs include tachycardia, hypertension, insomnia, hyperthermia, dilated pupils, panic attacks, seizures and aggressive behavior. In 2011, four DUID cases submitted to the laboratory for analyses involved designer stimulants. Case histories, signs and symptoms of impairment, results of field sobriety tests and toxicology results will be presented.

Materials and Methods: Blood evidence from the suspected DUID cases was submitted to the laboratory for alcohol and drugs analysis. The blood was tested for 17 drug categories including stimulants using ELISA. Two cases out of the four discussed in this paper were negative for stimulants by ELISA. In both cases the subject admitted to taking the drug Ecstasy. Further screening was ordered on these cases to identify other potential stimulant drugs. Specimens underwent solid-phase extraction followed by derivitization and were then analyzed by selected ion monitoring (SIM) and full scan gas chromatography/mass spectrophotometry (GCMS) for identification of stimulant drugs. Commercial standards were analyzed to confirm these new synthetic stimulants when available.

Results:

Case One: A 20-year-old male was stopped at a border patrol checkpoint. A blood ELISA screen was positive for 11-Nor-9-Carboxy-Delta-9-Tetrahydrocannabinol (THCA). Drugs confirmed by GCMS were Delta-9-Tetrahydrocannabinol (THC), THCA and methylone.

Case Two: A 20-year-old male was involved in an automobile crash. The blood ELISA screen was positive for THCA. Drugs confirmed by GCMS were THCA, midazolam and 1-benzylpiperazine (BZP).

Case Three: A 20-year-old male was stopped for speeding and erratic driving. The blood ELISA screen was positive for benzodiazepines, stimulants and THCA. Drugs confirmed by GCMS were THC, THCA, diazepam, nordiazepam, temazepam; 3,4-methylenedioxyamphetamine (MDA), methylone and mephedrone.

Case Four: A 51-year-old male was involved in an automobile crash. The blood ELISA screen was positive for stimulants and carisoprodol. Drugs confirmed by GCMS were doxylamine, meprobamate, carisopodol and citalopram. Presumptive methiopropamine (MPA) was found in a GCMS screen, but remains unconfirmed at this time.

Conclusion: Three of the subjects in this study reported ingestion of Ecstasy (3,4-methylenedioxymethamphetamine, MDMA) while the fourth subject stated that he had used cocaine and methiopropamine (MPA). Neither MDMA nor cocaine was identified in any cases; however, MDA was identified in one case that screened positive for stimulants by ELISA. Patient history should be reviewed carefully for recent stimulant use, and a second screen by GCMS targeting designer stimulants may be considered if the ELISA screen is negative.

Keywords: Driving, Synthetic Stimulants, Blood

P70

Characteristics and Application of Assays for Urine Creatinine, Oxidant and pH Determinations

Pao-Hsiu Liu[1,2], Meng-Yan Wu[1,3], Hei-Hwa Lee[4], Bai-Hsiun Chen[4], Chao-Jung Weng[5], Bud-Gen Chen[1], Mei-Han Huang[1], Huey-Lan Wang[1], **Ray H. Liu*[1]**; [1]Dept. of Medical Lab. Science and Biotechnology, Fooyin University, Kaohsiung, Taiwan, [2]Dept. of Lab. Medicine, Fooyin University Hospital, Ping-Tung, Taiwan, [3]Dept. of Lab. Medicine, Kai-Suan Psychiatric Hospital, Kaohsiung, Taiwan, [4]Dept. of Lab. Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan, [5]Axin Scientific Co., Long-Jing District, Taichung, Taiwan

Introduction: Specimen validity testing (SVT) has long been a mandatory test item in the U.S. federal workplace drug testing program (NLCP). This study evaluated the performance characteristics and effectiveness of assays available for the analysis of pH and the contents of creatinine and oxidants in urine specimens.

Objective: Findings derived from this study will be of reference value to laboratories searching for the most suitable reagents for their applications.

Methods: Assays included in this study came from four sources for creatinine; two for oxidant; and two for pH. Creatinine assays are based on Jaffe reaction; while the pH and oxidant assays are based on colorimetric method. Standards prepared in-house and provided by respective manufacturers were used as assay calibrators and controls. To help assess the assays' effetiveness, suspicious specimens (from operating laboratories) and performance testing (PT) samples (from NLCP) were also included in this study. Performance parameters studied included calibration characteristics and measurement precision. Precision data were further used as the basis to evaluate assays' (a) lower limits of detection; and (b) separations of responses between samples at the critical cutoff points (i.e., creatinine 2, 5, and 20 mg/dL; pH 4.5 and 9.0; nitrite 200 µg/mL and chromium 50 µg/mL) and samples with concentrations at their respective ±20% vicinities.

Results: Creatinine assays from all 4 sources appear to perform adequately at the 5.0 and 20 mg/dL level; while testing at the 2.0 mg/dL level may present a challenge to certain assays. pH assays from both sources perform adequately at the pH 2.30 to 11.65 range. Oxidant assays from different sources use different calibrators (nitrite or chromium VI) and perform adequately for the determination of the analyte that was used as the calibrator. However, a significantly higher result was observed when the assay was used for the analysis of the non-calibrator analyte. For example, the reagent using nitrite as the calibrator provided accurate result when it was used to analyze a test specimen containing nitrite; when it was used to analyze a test specimen containing 50 µg/mL chromium VI (equivalent of 200 µg/mL of nitrite), a much higher equivalent nitrite concentration was reported.

Conclusion: If the data derived from the analysis of the 597 suspicious specimens were interpreted based on the criteria mandated by NLCP, a very significant number of these specimens would have been reported as “adulterated", “substituted", or “diluted".

Keywords: Specimen Validity Testing, Creatinine, pH, Oxidant

P71

Presence of Codeine in 6-Acetylmorphine-Positive Urine Specimens in a Pain Population: An Improved Method for Heroin Testing

Sergey Latyshev*[1], Amadeo Pesce[1], Perla Almazan[1], Bridgit Crews[2], Eizabeth Gonzales[1], Charles Mikel[1], Cameron West[1], Robert West[1]; [1]Millennium Research Institute, San Diego, CA, USA, [2]Washington University St. Louis, St. Louis, MO, USA

Introduction: Illicit drug use is common in patients on chronic opioid therapy. At present, determination of patient heroin use is made by analysis for 6-acetylmorphine and is corroborated by the presence of morphine. A positive test causes concern because this may result in severe consequences for the patient beyond health risk. A second heroin-use marker could provide greater confidence to all concerned.

Objective: To determine the prevalence of codeine in 6-acetylmorphine-positive specimens and potentially provide support for the use of this marker in addition to 6-acetylmorphine and morphine to confirm heroin use.

Methods: Approximately 1,025,000 de-identified urine specimens from patients on opioid therapy for chronic pain were used in this retrospective study. Specimens were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) at Millennium Laboratories using validated methods previously described (Crew *et al*. Ther. Drug Monit. 2009;31:749-752). Specimens were analyzed for 6-acetylmorphine, morphine, and codeine. Positive specimens that had codeine listed as a medication were removed. The resulting 1,904 6-acetylmorphine-positive specimens were split into two groups: those with morphine values greater than 300 ng/mL (1,594 specimens) and those below 300 ng/mL (310 specimens). Those specimens with morphine below 300ng/mL were considered to have atypical 6-acetylmorphine metabolism.

Results: Of the 1,594 specimens positive for 6-acetylmorphine (cutoff concentration 10 ng/mL) and morphine (cutoff concentration 300 ng/mL), 1,459 specimens (92%) contained detectable codeine (cutoff concentration 50 ng/mL).

Conclusions: The prevalence of codeine in 6-acetylmorphine-positve specimens was greater than previously reported. The data support the concept that codeine is a significant impurity in heroin preparations and is readily observed by current LC-MS/MS methods. The finding of codeine in addition to 6-acetlymorphine and morphine may offer further support to the conclusion of heroin use.

Keywords: Heroin, 6-Acetylmorphine, Codeine, Patients with Pain, LC-MS/MS

P72

Concomitant use of Spice in Persons Prescribed Chronic Opioid Therapy for Pain

David Masters-Moore*, Sergey Latyshev, Charles Mikel, Amadeo Pesce, Cameron West, Elizabeth Gonzales, Robert West, Perla Almazan; Millennium Research Institute, San Diego, CA, USA

Introduction: The synthetic cannabinoids JWH018 and JWH073, commonly called Spice or K2, were marketed from 2009-2011 on the Internet and through retail outlets as providing a legal cannabis-like high while being undetectable by laboratories performing usual workplace testing. Once sold legally as 'incense', these agents are now banned due to serious public health risks. Because of the potential for harm and that illicit use may go undetected, the authors developed an assay method to detect the presence of these compounds and their metabolites in urine specimens.

Objective: To determine the prevalence of the use of illicit synthetic cannabinoids and THC in patients being treated for chronic pain with opioid therapy.

Methods: A retrospective analysis was conducted using 1,034,155 de-identified urine specimens from patients receiving chronic opioid therapy for treatment of pain tested using LC-MS/MS at Millennium Laboratories between March and December 2011. 53,799 urine specimens were tested for the presence of JWH018 and/or JWH073 and 708,109 specimens were tested for the presence of THC during this time. 48,178 of these specimens were tested for both illicit synthetic cannabinoids and THC.

Results: Of the 53,799 specimens, 421 (0.8%) specimens were positive for JWH018 and 483 (0.9%) for JWH073. Of the 708,109 specimens tested for THC, the positivity rate was 11.2%. For the group tested for both illicit synthetic cannabinoids and THC, there were 451 specimens (0.9%) that tested positive for Spice and 118 (0.2%) positive for both Spice and THC. Younger patients were more likely to have positive urine for the tested synthetic cannabinoid compounds; specimens which tested positive for synthetic cannabinoids had a mean age of 35 years compared with the average age of 50 years for the starting cohort.

Conclusions: About 1% of patients with chronic pain on opioid therapy tested positive for the synthetic cannabinoids, Spice/K2. The results suggest that younger patients may be more likely to use illicit designer drugs, such as Spice and K2. It appears uncommon that patients use both THC and Spice concomitantly. The observed concentrations of synthetic cannabinoids were lower than those of THC. We were unable to determine the cause for this, although we speculate that this could possibly be due to enhanced psychoactive effects of synthetic cannabinoids.

Keywords: Spice, THC, Marijuana, Chronic Pain, Opioid Therapy, Synthetic Cannabinoids, Urine Monitoring

P73

Determination of Endogenous Concentrations of GHB in Human Hair by LC/MS/MS

MacKenzie Willis*[1], Phillip Stout[2], Sarah Kerrigan[1]; [1]Sam Houston State University, Huntsville, TX, USA, [2]Texas Department of Public Safety Crime Laboratory, Austin, TX, USA

Introduction: Gamma-hydroxybutyrate (GHB) is both an endogenous neuromodulator and a central nervous system depressant capable of producing heavy sedation at recreational dosages. Due to the relatively short half life of the drug in blood and absence of useful metabolites; detection times in blood and urine are typically short (<12 hours). Delays in specimen collection following an allegation of sexual assault are not uncommon; and biological samples are not always collected within the necessary timeframe. Hair has been proposed as an alternative matrix due to the prolonged window of detection for most drugs. However; published data on endogenous GHB concentrations in hair are relatively sparse compared with other matrices.

Methods: A method capable of quantifying endogenous concentrations of GHB in human head hair was developed; optimized and validated using liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS). To avoid lactonization of the drug, hair was digested under alkaline conditions and GHB was isolated using a simple liquid-liquid extraction. LC/MS/MS was performed using atmospheric pressure chemical ionization (APCI) in the negative mode; multiple reaction monitoring (MRM) and deuterated internal standard (GHB-D6). In an IRB approved study, 200 human volunteers that had not used GHB; provided head hair from the posterior vertex. These samples were used to determine endogenous GHB concentrations in hair samples.

Results: Optimum digestion conditions were achieved using 1mL sodium hydroxide (0.1M) at 56C. Digests were filtered using a 0.45 µm filter containing a hydrophilic membrane (polyethersulfone) prior to extraction. Precision and accuracy at 25 ng/mg (n=4) were 2% and 105%, respectively. Beta-hydroxybutyrate (BHB), alpha-hydroxybutyrate (AHB), gamma-butyrolactone (GBL) and 1:4 butanediol (BDL) did not produce an interference. There was negligible ion suppression or enhancement from the matrix and the limits of detection and quantitation of the assay were 0.2 and 0.4 ng/mg. The mean and median GHB concentration in hair were 1.9 and 1.5 ng/mg, respectively (n=33). The range was 0.4-6 ng/mg (n=33). Additional data from the remainder of the samples is forthcoming.

Conclusions: LC/MS/MS was used to develop and optimize a highly sensitive procedure for the detection and quantitation of endogenous GHB concentrations in hair. Interpretation of GHB concentrations in biological matrices is complicated because one must be able to differentiate endogenous from exogenous administration in criminal casework. Understanding the range of endogenous GHB concentrations in human head hair will help determine how feasible it is to interpret quantitative GHB data from a complicated matrix such as hair.

Keywords: Gamma-Hydroxybutyrate (GHB), Hair, Endogenous, LC/MS/MS

P74

Screening of 300 Drugs in Human Blood Utilizing Second Generation Exactive High Resolution Accurate Mass Spectrometer and ExactFinder Software

Kristine Van Natta*, Xiang He, Marta Kozak; Thermo Fisher Scientific, San Jose, CA, USA

Introduction: Forensic scientists and toxicologists need to search for many different compounds in samples of human blood. Endogenous matrix components and the wide variety of possible compounds make the task daunting. The second generation Exactive high resolution accurate mass spectrometer with new broad mass filtering capabilities and fast polarity switching enables identification of compounds in a wide chemical space with minimum interference from endogenous compounds. Additionally, full scan data allows for retrospective analysis of data for previously unknown compounds.

Methods: Standards are prepared by spiking compounds into blank human whole blood at concentrations of 10, 100 and 1000 ng/mL. Samples and standards are processed by precipitating a 50-µL aliquot of blood with 150 µL of $ZnSO_4$/MeOH containing internal standard, centrifuging and diluting. An aliquot is analyzed by a gradient HPLC method using a Thermo Hypersil GOLD 5 µm particle size, 100 x 3 mm column. Mobile phase is 10 mM ammonium acetate with 0.1% formic acid in water (A) and methanol (B). The compounds are detected on a second generation Thermo Exactive mass spectrometer equipped with an APCI probe. The instrument is operated in alternating positive and negative full-scan and all-ion fragmentation mode with fast polarity switching with a resolution of 35,000 to 70,000 (FWHM) at m/z 200. Chromatograms are reconstructed with a mass tolerance of 5 ppm. Compounds are identified based on exact mass, retention time, fragments and isotopic pattern using ExactFinderTM software.

Results: Many different classes of compounds have been identified at cutoff concentrations of 5-50 ng/mL. Screening results from this assay agreed with results obtained from immunoassay on donor samples. Isomeric compounds such as morphine and hydromorphone, methamphetamine and phentermine, are baseline separated with chromatography. No carryover was seen during analysis. Fast polarity switching enabled identification of both positively and negatively charged species in one analytical run. Average mass accuracy was ~3 ppm.

Conclusion: Faster scan rate on the second generation Exactive gave more scans across an individual peak than on previous Exactive instrument. The new broad mass filtering reduces the high mass transmission of endogenous components such as proteins and phospholipids thus providing better sensitivity and reduced noise. Combining these new features gives high confidence identification of 300 drugs representing many drug classes in whole blood using high resolution accurate mass approach.

Keywords: HRAM, Whole Blood, Screening, Forensic Toxicology

P75

Quantitative Analysis of Six Opioids in Urine Using Full Scan LC-MS^2 and MS^3 Linear Ion Trap Technology

M.Kozak*[1], J.Horner[1], Kristine Van Natta[1], M.Moseley[1], M.P. George[2]; [1]Thermo Fisher Scientific, San Jose, CA, USA, [2]Alere Toxicology Services Inc., Richmond, VA, USA

Background: Traditionally GC-MS instruments or triple quadrupole mass spectrometers coupled with liquid chromatography are used for quantitative analysis of opioids in urine samples. Implementation of LC-MS techniques will increase since new SAMHSA regulations in 2011 allow for the use of LC-MS.

Objective: We investigated the performance of a new method with a linear ion trap Velos Pro™ collecing full scan MS^2 and MS^3 spectra in a toxicology lab.

Method: Urine samples were spiked with internal standards (deuterated analogues), subjected to enzymatic hydrolysis and either processed with SPE method or diluted 20-folds with DI water. 20 µL was injected onto a Hypersil GOLD PFP column. Analytes (morphine, hydromorphone, oxymorphone, codeine, hydrocodone, oxycodone) were separated in 5.7 min under gradient conditions using 5 mM ammonium acetate/0.1% FA in DI water as mobile phase A and 5 mM ammonium acetate/0.1% FA in MeOH as mobile phase B. MS analysis was performed with linear ion trap Velos Pro equipped with heated ESI source. Full scan MS2 data were collected for all analytes except two for which full scan MS3 data were collected to obtain more specific fragments. For each analyte two fragments were monitored and the ion ratio was calculated for confirmation.

Method Validation: The calibration standards in human urine (range 50-2000 ng/ml) and QC samples (at concentrations 225, 375, 735, 2259 ng/mL) were prepared in-house. Upper limit of linearity was set to 2000 ng/mL. Method imprecision, LOD and LOQ were obtained by processing and analyzing each calibration standard and each QC sample in five replicates in five different analytical runs. Results obtained with Velos Pro were correlated with results obtained on both GC-MS and LC-triple quadrupole mass spectrometer. Matrix effects in samples processed with dilution procedure were evaluated by comparing signals of morphine and codeine in standards prepared in DI water to spiked urine from 100 donors. Opioids-positive samples from 70 donors were analyzed and data from different methods were correlated. With the method all opioids can be distinguished or chromatographically separted to eliminate interferences.

Results: The calibration range for all analytes was 50-2000 ng/mL with R^2>0.99. The LODs and LOQs calculated using Linest function were between 1.5-5 ng/mL and 11-30 ng/mL respectively. Analyte peak area imprecision (%RSD) calculated for all standards and QCs was less than 14.4%. Internal standard peak area imprecision was less then 12.0%. R^2 of correlation between GC-MS donor urine results and Velos Pro LC-MS results was 0.95. R^2 of correlation between Velos Pro donor urine results and triple quadrupole results was 0.98. Matrix effects (recovery outside 50-130% range calculated against water spiked samples) were observed in 4% of urine samples spiked with codeine and were not observed in samples spiked with morphine.

Conclusion: The method was successfully validated for implementation in a SAMHSA and toxicology laboratory. The linear ion trap method showed comparable results to traditional GC-MS and LC-triple quadrupole method.

Keywords: SAMHSA Regulations, Opioids in Urine, Quantitative Analysis of Opioids

P76
Expanding Urine Testing for Synthetic Cannabinoids

Sherri L. Kacinko, Joseph W. Homan, **Donna M. Warrington***, Barry K. Logan; NMS Labs, Willow Gtove, PA, USA

Introduction: Synthetic cannabinoids continue to be a significant analytical challenge to the toxicology community. The chemicals used in herbal incense products are constantly changing in an attempt to bypass laws controlling specific analytes. This requires constant re-evaluation of current methods to make sure the most commonly used analytes are covered in testing. Based on data collected from analysis of herbal incense products and blood testing results, a liquid chromatography-positive ion electrospray ionization- tandem mass spectrometry analysis for the qualitative identification of metabolites of nine synthetic cannabinoids was developed and validated.

Methods: Fortified urine specimens (1 mL) underwent enzymatic hydrolysis followed by liquid-liquid extraction with 3 mL methyl t-butyl ether. Chromatographic separation was achieved in 13.5 minutes using a Pinnacle DB Biphenyl (100 x 2.1 mm; 1.9 µm) column and a Waters Acquity UPLC with gradient elution. A Waters Acquity TQD mass spectrometer was used to monitor two transitions for each analyte of interest and internal standard. Validation paramenters include precision around the reporting limit; sensitivity; specificity; interfering substances; matrix effect; extraction efficiency; stability and robustness.

Results: Validation of decision concentration acceptability, precision around the decision point, extraction efficieny and matrix affect are complete at this time.

Conclusions: All tested parameters are acceptable. The cutoff concentration of each analyte was verified by analyzing 40 replicates of the cutoff control and determining the percent positive. The precision around the cutoff is ± 25% for all analytes (71.8% confidence). Validation of sensitivity, specificity, interfering substances, stability and robustness are underway.

Analyte	Cutoff Conc. (ng/mL)	% Positive at Cutoff	Extraction Efficiency,%	Matrix Effect, %
Desmethyl-4-OH-RCS-4	0.5	50	85	-45
Desmethyl-4-OH-JWH-081	0.5	50	85	63
Desmethyl-5-OH-JWH-081	0.5	47.5	85	84
4-OH-JWH-250	0.5	55	91	-7
4-OH-JWH-073	0.1	47.5	86	-20
3-OH-JWH-073	0.1	47.5	91	10
4-OH-JWH-018	0.1	45	92	0
4-OH-AM-2201	0.2	45	92	1
5-OH-JWH-018	0.2	45	93	20
5-OH-JWH-019	0.2	55	91	15
6-OH-JWH-019	0.2	52.5	92	14
4-OH-JWH-122	0.2	52.5	92	10
5-OH-JWH-122	0.2	55	92	11
4-OH-JWH-210	0.2	57.5	91	10
5-OH-JWH-210	0.2	40	85	-45

Keywords: Synthetic Cannabinoids Metabolites, Urine

P77

Automated Extraction of Benzodiazepines in Urine Using Dispersive Pipette Extraction (DPX) Tips with a Tecan Robotic System

Paul V. Robandt[1], Christopher L. Powell[1], Huong M. Bui[1], William E. Kemnitzer[2], William E. Brewer[2], **Claire Collins***[3], and William T. Murphy[2], [1]Navy Drug Screening Lab, San Diego, CA, USA, [2]DPX Labs, LLC, Columbia, SC, USA, [3]Hummingbird Research and Development, Natick, MA, USA

Introduction: Analysis of benzodiazepines in urine is commonly performed using enzymatic hydrolysis followed by solid-phase extraction (SPE). SPE methods are often performed manually though numerous types of automated SPE platforms currently exist. An ideal way to perform SPE is to use the same robotic platform that is being utilized for the enzymatic hydrolysis step, especially for high throughput applications. High throughput robotic systems utilize pipette tips for handling liquid samples so it would be ideal to incorporate pipette tips for performing SPE. In this study; dispersive pipette extraction (DPX) tips are used with a Tecan robot for extracting 5 common benzodiazepines in urine.

Objective: To develop a method of extracting benzodiazepines in urine using a Tecan robot with DPX tips.

Methods: The benzodiazepine standards were purchased from Cerilliant Corp and these standards include nordiazepam; lorazepam; temazepam; oxazepam; alpha-hydroxy-alprazolam; and deuterated analogues of each target compound. Automated DPX extractions were performed using a Tecan EVO-100 liquid handling platform. The DPX tips were made using a new Patent pending technology to ensure “dispersive" extractions occur reproducibly during the automated extractions. The LC/MS/MS system was a Waters TQC MS-MS with Acquity pumps and autosampler. Urine samples (500 µL) were first enzymatically hydrolyzed using β-glucuronidase and buffer (pH 5). The DPX tips (Hb-T (WAX)) were first conditioned with 30% methanol in water. After dispensing the solution to waste, the sample solution was aspirated into the DPX tips, mixed by aspirating air bubbles, and then dispensed into the corresponding sample vial. The extraction process was repeated. The sorbent was subsequently washed with 500 µL of DI water by aspirating, mixing and dispensing to waste. The benzodiazepines were eluted by aspirating 500 µL of methanol, mixing by aspirating with air bubbles, and then dispensing into clean vials. The Tecan robotic system processes eight samples simultaneously in approximately seven min.

Results: Spiked urine samples were extracted using the automated method developed with the Tecan robot and DPX tips. Recoveries of all 5 benzos was greater than 70% with %RSDs of less than 8%. The very good reproducibility for the analyses required optimization of the design of the DPX tips which included improved flow characteristics and assurance of “dispersive" SPE by using material to break up the sorbent particles. In addition to the spiked samples, urine case samples were also analyzed. Fifty case samples that were already analyzed and confirmed were processed with this method. No statistical difference was found with this current method with the results from the previous validated method. The main advantage of the robotic procedure is that the samples are processed from start to finish with automation without manual handling of the samples. Other advantages include speed with eight samples being ready for analysis in under eight minutes and use of low volumes of organic solvent. Only approximately 0.6 mL of methanol is used for the extraction of each sample.

Keywords: Dispersive Pipette Extraction, Benzodiazepines, LC/MS/MS

P78

Quantitation of Antidepressants (Citalopram, Venlafaxine and Paroxetine) in Serum by LC-MS/MS

Paula M. Ladwig, **Loralie J. Langman***; Mayo Clinic, Rochester, MN, USA

Background/Introduction: Citalopram and paroxetine are selective serotonin reuptake inhibitors (SSRIs) approved for the treatment of wide variety of psychological disorders, including major depression and obsessive-compulsive behavior. Venlafaxine is a serotonin and norepinephrine reuptake inhibitor (SNRI) also approved for a wide variety of psychological disorders.

Objective: We present here an accurate and robust method that identifies and quantitates citalopram, desmethylcitalopram, paroxetine, venlafaxine, and desmethylvenlafaxine in serum, for the purpose of monitoring clinical response and possible toxicity, optimizing drug dosage, and assessing compliance.

Methods: Drugs were extracted from serum by SPE. Briefly, 200µL of patient sample was combined with 50µL of 50ng/mL deuterated internal standards. Oasis HLB 1cc Cartridges were conditioned with 1mL methanol then washed with water. Sample with IS were placed on the column and washed with 1mL 5% methanol. The drugs were eluted with 1mL 80% methanol. Eluent was dried down then reconstituted in 0.2mL 5% methanol; 20µL was analyzed using an API 3200 LC-MS/MS with a Zorbax Eclipse XDB C18 (4.6 mm by 50 mm, 1.8 µm) column. Separation was achieved using a flow of 0.4mL/min of 90% 20 mM ammonium acetate 2% acetonitrile in water (A) and 10% 0.1% formic acid in acetonitrile (B). Concentration of B increased over 2.5min and then held steady for 1min at 50%. The column was cleaned with 100% B and then re-equilibrated for the next injection.

Results: The AMRs were as follows; citalopram 5-500ng/mL, desmethylcitalopram 8-800ng/mL, venlafaxine and desmethylvenlafaxine 5-1000ng/mL, and paroxetine 10-500ng/mL. Within- and between-run imprecision were less than 10% across the analytical range. Comparison samples were analyzed and demonstrated good agreement. Analyte and extract stability, ion suppression, carryover, and interference studies were also performed.

CITALOPRAM				
	N	**Mean**	**SD**	**%CV**
Intra-assay	20	23.4	1.16	5.0
	20	61.1	1.98	3.2
	20	293	9.91	3.4
Inter-assay	20	9.3	0.64	6.8
	20	63.5	2.93	4.6
	20	300	21.55	7.2
Accuracy	66	y=0.92x+1.69; R^2=1.00		
VENLAFAXINE				
	N	**Mean**	**SD**	**%CV**
Intra-assay	20	56.9	1.97	3.5
	20	110	5..44	5.0
	20	354	10.46	3.0
Inter-assay	20	105	2.82	2.7
	20	123	5.38	4.4
	20	365	14.29	3.9
Accuracy	52	y=0.97x+6.91; R^2=0.99		
PAROXETINE				
	N	**Mean**	**SD**	**%CV**
Intra-assay	20	15.3	0.97	6.3
	20	35.5	1.90	5.3
	20	98.5	5.37	5.5
Inter-assay	20	44.5	3.27	7.4
	20	119	9.74	8.2
	20	437	26.12	6.0
Accuracy	29	y=1.10x-6.28; R^2=0.99		

DESMETHYLCITALOPRAM				
	N	**Mean**	**SD**	**%CV**
Intra-assay	20	39.3	2.51	6.4
	20	126	7.46	5.9
	20	826	17.48	2.1
Inter-assay	13	38.1	2.78	7.3
	20	158	6.10	3.9
	20	802	24.59	3.1
Accuracy	18	y=0.95x+3.08; R^2=1.00		
DESMETHYLVENLAFAXINE				
	N	**Mean**	**SD**	**%CV**
Intra-assay	20	119	6.27	5.3
	20	229	7.26	3.2
	20	526	15.25	2.9
Inter-assay	20	100	3.13	3.1
	20	230	9.96	4.3
	20	525	19.54	3.7
Accuracy	51	y=0.99x-4.79; R^2=0.98		

Conclusion/Discussion: The validation performed demonstrated acceptable performance.

Keywords: Quantitation of Antidepressants, Citalopram, Venlafaxine, Paroxetine

P79
A Suspected Drug Facilitated Crime Investigated by Hair Analysis

Sys Stybe Johansen*, Marie Katrine Klose Nielsen; Section of Forensic Chemistry, Department of Forensic Medicine, Faculty of Health Sciences, University of Copenhagen, Denmark

Introduction: The girlfriend of an elderly man attempted to murder him. After eating a meal the man suddenly felt drowsy and lay down. Shortly thereafter, the girlfriend attacked him with a knife but he managed to escape. Since the girlfriend was a trained nurse and was prescribed several drugs due to her status as a terminal cancer patient (for several years) the police contacted the Forensic Chemistry Laboratory on suspicion of a drug facilitated crime one week after the incident.

Objective: Segmented hair analysis was applied to identify the use of drugs in a drug facilitated crime case with delayed sample collection.

Methods: A hair sample was taken from the man one month after the incident. The hair was cut into three segments, washed, dissolved and analyzed for drugs and drugs of abuse by UPLC/TOF and LC/MS/MS. Positive findings were confirmed by UPLC/MS/MS.
A preserved blood-alcohol sample taken two hours after the incident was later recovered for further forensic analysis. Similar analyses of the blood sample were performed.

Results: Findings in hair and whole blood.

	Hair (ng/mg)			Blood
	(0-1.5 cm)	(1.5-3.0 cm)	(3.0-4.5 cm)	(mg/kg)
Morphine	0.29	0.06	0.06	0.085
Diazepam	0.053	0.038	0.055	0.17
Desmethyldiazepam	0.084	0.022	0.032	0.068
Zopiclone	0.02	0.01	0.02	0
Lidocaine	29	54	74	0.16
Monoethylglycinxylided	0.24	0.34	0.49	0.021

The finding of drugs in the inner hair segment (0-1.5 cm) was consistent with the drugs found in the blood sample. The high level of morphine in blood and hair was remarkably since morphine was only prescribed to his girlfriend. The low levels of zopiclone, diazepam and its metabolite desmethyldiazepam in hair concur with a small intake of these drugs within a 4½ month period as reported by the man. The origin of lidocaine was unknown.

Conclusion: The case illustrates that hair is a valuable forensic specimen in situations where natural processes have eliminated the drug from typical biological specimens because of delayed sample collection. It was possible to verify the findings of the hair analysis with a recovered blood sample taken from the victim shortly after the incident.

Keywords: Hair, Drug Facilitated Crime, Opioid

P80

Examining the Postmortem Redistribution Effects of Alprazolam in Blood

Samantha S. Tolliver*[1], W. Lee Hearn[2] , Kenneth G. Furton[3]; [1]ExperTox, Inc, Deer Park, TX, USA, [2]Miami-Dade Medical Examiner, Miami, FL, USA, [3]Florida International University, Miami, FL, USA

Introduction: Alprazolam is one of the most frequently prescribed medications in the United States. It is mostly used to treat anxiety-related illnesses; but its omnipresence (whether through licit or illicit use) makes it among the more commonly encountered prescription medication in postmortem toxicology cases. When interpreting postmortem results; the impact of postmortem redistribution (PMR) must be considered. Alprazolam has an Apparent Volume of Distribution (Vd) that is relatively low (0.9 - 1.3 L/kg). This would suggest that PMR may not be a major factor for interpretation.

Objective: To determine if and when PMR of alprazolam would be a critical factor in postmortem drug interpretation.

Methods: Ten medical examiner cases, submitted with their respective antemortem specimens, were evaluated in an effort to determine the redistribution potential of alprazolam. The samples were prepared by liquid-liquid extraction and analyzed both by Gas Chromatography-Electron Capture Detector and Liquid Chromatography-Mass Spectrometry. When interpreting the results, many factors were considered, including the postmortem to antemortem ratio (PM/AM), central to peripheral ratio (C/P), postmortem interval and the antemortem to death interval.

Results: The lower limit of quantitation for the alprazolam assay was 0.01 mg/L. The case samples had concentrations ranging from 0.01 – 0.48 mg/L. The median PM/AM ratio was 2.11 (range 0.08 – 4.0). The median C/P equaled 0.9 (range: 0.5 – 1.3).

Discussion/Conclusion: Typically when examining PMR ratio calculations, those values that are greater than 1.0 indicate some degree of redistribution. The data for these cases gave conflicting ratios. The C/P suggests a lack of redistribution, while the PM/AM suggests the opposite. A closer look at the data as a whole indicates that alprazolam does not appear to have a propensity for PMR. This is in agreement with the relatively low apparent volume of distribution. However, a trend was identified. Under certain circumstances, namely a short antemortem to death interval ($\leq$ 90 minutes) coupled with a long postmortem interval ($\geq$ 45.5 hours) redistribution is more likely to occur.

Keywords: Postmortem Redistribution, Alprazolam and Postmortem Interval

P81
Fatal Case Study of Acute Yohimbine Intoxication

Colin C. Anderson*, Nicole M. Harre, John X. Hu, Norman A. Wade; Office of the Medical Examiner, Forensic Science Center, Phoenix, AZ, USA

Introduction: Yohimbine is an indole alkaloid rarely seen in forensic casework that has important implications due to its varied applications. It has been approved for use in the United States to treat erectile dysfunction; although its effectiveness has been highly controversial. Yohimbine has also been abused on the streets as an aphrodisiac and hallucinogen; while more recently it has risen in the body-building community for its lipolytic and sympathomimetic effects. Therapeutic levels of the drug in blood range from 40-400 ng/mL after an average oral dose of 5-15 mg. Overdoses leading to neurotoxic effects have been seen from doses of 200 mg to 5 g resulting in blood concentrations up to 5000 ng/mL. Symptoms reported with Yohimbine overdose include anxiety; drowsiness; disorientation; tremors and seizures with higher doses. Little data of Yohimbine levels in blood is available, and no fatal cases were observed. In this case, a 23-year-old male body builder with a history of mixing his own energy and protein drinks along with a medical history of low testosterone and steroid use collapsed outside of a fitness center in Gilbert, AZ. Emergency personnel responded after he called a friend stating he “thought he was dying" and “may have put too much caffeine in his energy drink", though he was known by family to be meticulous in his measurement. He presented to the ER with seizures and elevated vitals and was pronounced dead within hours. Through investigation, jars of Yohimbine and Caffeine powder were among supplements recovered from the decedent's residence. Autopsy findings included cardiomegaly (525 g), pulmonary edema, and congestion. Multiple post-mortem samples including vitreous fluid, iliac blood, urine and bile were drawn and sent to toxicology for testing along with the admission specimens.

Objective: To inform the forensic science community on the background, use and abuse, detection, and potentially fatal blood levels of a rarely seen supplement.

Methods: Volatile analysis was performed on both vitreous fluid and hospital blood using an Agilent 7890 GC/FID. EIA was performed on hospital specimens followed by quantitation of any positives. A basic drug screen was performed on urine using an Agilent 6890 GC/NPD followed by confirmation on an Agilent 5973 GC/MS following a liquid-liquid extraction. Due to limited sample, hospital blood was only analyzed on the 5973 GC/MS. An acidic/neutral screen was performed on post-mortem blood using an Agilent 5975 GC/MS with a DB-17 column following an SPE extraction.

Results: Toxicology testing on hospital specimens yielded negative results for volatiles and EIA screen (Barbiturates, Benzodiazepines, Benzoylecgonine, Opiates, Methamphetamine, Fentanyl, and Oxycodone). The acidic/neutral screen on was unremarkable, while the basic drug screen confirmation revealed the presence of Caffeine, Diphenhydramine, and Yohimbine (matches to AAFS, SWGDRG, and WILEY libraries). White powder collected from Gilbert PD in the jar labeled “Yohimbine" was also analyzed and compared to the blood results. Iliac Blood was sent to National Medical Services Laboratory, Willow Grove, Pennsylvania (NMS) for Yohimbine quantitation by HPLC; which was subsequently reported to be 7.40 mg/L.

Conclusion/Discussion: Cause and Manner of death was determined to be an accident by acute Yohimbine intoxication. Presently; the case is being investigated as a possible homicide due to allegations of poisoning from the spouse by family members.

Keywords: Yohimbine, Fatality, Dietary Supplement

P82

Toxicology in Emergency Room in the Valais Hospital between 2007 and 2011

Nicolas Donze*[1], Luc Fornerod[2], Arnaud Chiolero[2], Rene Bonvin[2], Michel F. Rossier[1] , Marc Augsburger[3]; [1]Central Institut, Valais Hospital (ICHV), Grand Champsec, Switzerland, [2]Observatoire valaisan de la sante (OVS), Grand Champsec, Switzerland, [3]University Center of Legal Medicine, Lausanne-Geneva, Switzerland

Introduction: Inspired by the Drug Abuse Warning Network, which presents an estimation of drug-related visits to hospital emergency departments (EDs) in USA, we assessed the number of drug-related visits to hospital emergency departments in one canton of Switzerland, in Valais, where about 300,000 people are living.

Objective: This study aims at characterizing the drug-related visits to Valais Hospital emergency departments (EDs) between 2007 and 2011 based on data from the Central Institute laboratory and from the Observatoire valaisan de la sante (OVS).

Methods: All patients entered into the ED since 2007 to 2011 in the Valais Hospital's sites of Brig; Visp; Sierre; Sion (pediatric and general ED) and Martigny were considered. Two kinds of requested toxicological analyses were analyzed: screenings of drugs of abuse (DOA: acetominophen; amphetamine; metamphetamine; barbiturates; benzodiazepines; cannabinoids (THC); cocaine; methadone; opiates; PCP; TCA) by immunological test (Triage; Biosite) in urine and measure of ethanol in serum with colorimetric method (Cobas 6000 Roche). The results for DOA test were qualitative (positive or negative) and the result for alcohol was quantitative (g/kg) (LOQ = 0.15 g/kg).

Results: The total number of ED visits between 2007 and 2011; increased from 64725 to 69867 (8 %). The number of requests for DOA or/and alcohol increased from 1922 to 2381 (24 %) as well. It has been observed that the percentage of requests for DOA or/and alcohol among ED visits presents a small increase (3.0 % to 3.4 %). The number of requests for DOA only presents an increase of 71 % between 2007 and 2011. The two drugs most frequently detected are the THC and the benzodiazepines. The result for alcohol is positive in about 70 % of the cases.

Conclusions: In Valais Hospital, the percentage of drug-related visits to hospital emergency increased from 3.0 % to 3.4 % of all ED visits between 2007 and 2011. Alcohol was the drug most frequently investigated and detected. The number of DOA investigations increased between 2007 and 2011, and benzodiazepines and THC were the two drugs the most frequently found. The rate of positivity was stable during this period.

Keywords: DOA, Blood Alcohol, Emergency Departments

P83

Assessment of Different Dried Blood Spot Cards for Opioids, Cocaine, Amphetamines, Benzodiazepines and THC Analysis

Ana de Castro[*1,2], Marta Concheiro[1], Elena Lendoiro[1], Angelines Cruz[1], Manuel López-Rivadulla[1]; [1]Instituto de Ciencias Forenses, Universidad de Santiago de Compostela, Santiago de Compostela, Spain, [2]Cienytech, S.L., Santiago de Compostela, Spain

Introduction: Dried blood spot (DBS) is a blood sampling procedure of increasing interest. Biohazard risk is minimum, transport and storage easy, and many analytes gain stability.

Objective: To assess five DBS cards: BondElut DMS (Agilent), FTA DMPK-A, DMPK-B, DMPK-C and Classic (Whatman), for the analysis of opioids (morphine, codeine, 6-acetylmorphine), amphetamines (amphetamine, metamphetamine, MDA, MDMA, MDEA), cocaine (cocaine, benzoylecgonine), benzodiazepines (alprazolam, clonazepam, oxazepam, nordiazepam, lorazepam, flunitrazepam, diazepam) and THC in blood samples.

Methods: DBS cards were fortified with 50µL blood spiked at 10ng/mL for all compounds and dried at room temperature for 3h. Preliminary comparison of different solvents was performed by extracting each card type in replicate with 3mL of one of the following solvents: methanol (MeOH), acetonitrile (ACN), ethylacetate/hexane (50:50), dichloromethane/2-propanol (50:50), phosphate buffer pH6, borate buffer pH9 (except for DMPK-B and Classic) and formic acid 2% (except for Classic). After shaking 30min, solvents were transferred to a clean tube. Aqueous buffers were further extracted with 3mL ethylacetate/hexane (9:1) after alkalinization. Samples were evaporated to dryness, reconstituted in formic acid 0.1%/ACN (90:10) and analyzed by LC-MS/MS. In a second experiment, extraction solvent was further optimized comparing MeOH/ACN in different proportions using DMS cards (n=2). Matrix effect, extraction and process efficiency (n=2) were determined employing DMS, DMPK-A, DMPK-B and DMPK-C cards and 3mL MeOH/ACN (80:20) extraction solvent.

Results: Morphine, codeine and 6-acetylmorphine best results were obtained with DMS and DMPK-C cards employing MeOH, borate buffer or formic acid 2% as extraction solvent. DMS, DMPK-B and DMPK-C cards allowed the best results for amphetamines using any of the tested solvents. Best results for cocaine and benzoylecgonine were achieved employing DMS and DMPK-C cards with MeOH, and for benzodiazepines, using DMS and DMPK-C cards with phosphate buffer, followed by borate buffer, formic acid 2% and MeOH. For THC, only DMPK-A and Classic cards allowed a sensitive and reproducible detection, with the highest signal obtained with dichloromethane/2-propanol; however, DMPK-A and Classic cards did not allow detection of opiates and amphetamines with any of solvents, and led to the worst sensitivity for cocaine and benzodiazepines (Table 1). Comparing different MeOH/ACN proportions employing DMS cards, MeOH/ACN (80:20) produced the best results for all the analytes. Matrix effect for opioids, amphetamines and cocaine was -100% with DMPK-A cards, and -6 to -54% and -7 to -86% with DMS and DMPK-C cards, respectively; extraction efficiency with DMS and DMPK-C range from 22-107% and 50-103%, respectively. For benzodiazepines, matrix effect with DMS, DMPK-A, DMPK-B and DMPK-C cards ranged from -13 to 88%, -76 to -91%, -45 to -81% and -6 to -73%, respectively; extraction efficiency ranged from 41-76%, 85-111%, 81-103%, 76-95%, respectively. For THC, no signal was obtained when the analyte was fortified in a clean tube and, therefore, matrix effect could not be calculated; DMPK-A cards allowed the highest extraction efficiency (104%), followed by DMPK-B (47%) and DMS (7.5%). THC was not detected in DMPK-C cards.

Conclusion/Discussion: DMS and DMPK-C cards employing MeOH/ACN (80:20) and MeOH allowed the highest sensitivity for the simultaneous detection of all the analytes, except THC, which could only be detected using DMPK-A and Classic cards.

Keywords: Dried Blood Spot (DBS), Drugs of Abuse, Benzodiazepines

P84

Hair Analysis for Opioids by Liquid Chromatography / High Resolution Mass Spectrometry

Madeline A. Montgomery*, Roman P. Karas, Marc A. LeBeau; FBI Laboratory, Quantico, VA USA

Background/Introduction: Opioids are a class of drug often encountered in forensic toxicological casework. They may be drugs of interest in driving under the influence cases, cases of suspected drug-facilitated sexual assault and post-mortem investigations. Hair analysis to detect long-term exposure to opioids may aid in an investigation of drug abuse or misuse. Liquid chromatography / high resolution mass spectrometry provides both the specificity and sensitivity required for this analysis.

Objective: The objective of this study was to validate a method for the analysis of hair samples for morphine; 6-acetyl morphine; oxycodone; hydrocodone; hydromorphone; codeine; fentanyl; meperidine; tramadol and methadone.

Methods: Hair samples were decontaminated with a series of solvents before analysis. Samples were ground to a powder using a cryogrinder. The resulting powder was extracted overnight with methanol. Methanol extracts were taken to dryness using an evaporator and reconstituted in water for solid phase extraction. Final extracts were analyzed by liquid chromatography / high resolution mass spectrometry.

Results: This method was fully validated according to laboratory requirements. The following parameters were evaluated as part of the validation experiments: accuracy; calibration model; carryover; interferences; internal standard crosstalk; ion suppression/enhancement; limit of detection; lower limit of quantitation; precision; processed sample stability; recovery and selectivity.
All parameters were found to be within acceptable criteria. Accuracy and precision were measured at three control levels on five days and were within expected tolerances (20% and 15%; respectively). A linear calibration model with 1/x weighting was found to provide linearity across the linear range of 100 - 3000 pg/mg for all analytes except for fentanyl, which was successfully validated from 20 - 300 pg/mg. Ion suppression values varied from analyte to analyte, but is accounted for by using isotopically labeled internal standards. Processed samples were found to be stable for as long as seven days post extraction for all analytes except for methadone. Recovery; in general, was poor. However, since LODs were adequate and isotopically labeled internal standards are used, this was considered acceptable. Numerous common drugs and metabolites were found not to interfere with the method. Additionally, no interferences were identified in 11 blank hair samples.

Results from two positive forensic cases will be reported: The first involved testing of the hair from a child that had allegedly died of a morphine overdose. Investigators wanted to know if the child had been exposed to opioids before the overdose that led to his death. Both morphine and oxycodone were identified in the child's hair, suggesting prior exposure. The second case involved the testing of hair from a medical professional accused of abusing narcotics. Both fentanyl and hydrocodone were identified in this individual's hair.

Conclusion/Discussion: This method was fully validated for the quantitative analysis of opioids in hair samples. It has since been applied to numerous forensic cases with both positive and negative findings.

Keywords: Hair Analysis, Opioids, Liquid Chromatography / High Resolution Mass Spectrometry

S06 - Replacement

The Need To Establish A Quantitative Criterion For Distinguishing *l*-Methamphetamine From Inhaler Use From Abuse Of Illicit *l*-Methamphetamine

Theodore F. Shults, JD, MS. Chairman of the American Association of Medical Review Officers, Research Triangle Park, NC, USA

Introduction: This presentation proposes that a quantitative criterion needs to be established for total *d* + *l* methamphetamine levels in urine in addition to and/or alternative to chiral analysis to distinguish illicit methamphetamine from a claim of inhaler use. There are three known sources of *l*-methamphetamine in urine: the use of nasal inhalers, (Vick's Nasal Inhaler – sold under a number of brands), metabolites of the MAOI drug Selegiline (*l*-deprenyl, Eldepryl, Emsam, Zelapar) and clandestinely manufactured methamphetamine. Selegiline can be readily verified by prescription, OTC drugs cannot.

l-methamphetamine is permitted by the FDA and DEA solely for over-the-counter distribution as a constituent of a nasal inhaler. *l*-methamphetamine acts as peripheral vasoconstrictor *l*-methamphetamine is the levorotary (R-enantiomer) of methamphetamine. As a mirror image of *d*-methamphetamine the *l*- isomer produces an identical GC/MS profile in terms of retention time and ions. The HHS and DOT guidance to Medical Review Officers is to order a chiral analysis of a disputed methamphetamine specimen[1.] Chiral analysis is, however, by itself inadequate to distinguish clandestinely manufactured methamphetamine from a claim of OTC inhaler use. The DEA laboratories and some state crime labs have reported confiscated methamphetamine samples have been found that are up to 99% *l*-methamphetamine[2]. The putative explanation is, this is a result of alternative clandestine manufacturing techniques.

Methods: In May 2008 MRO*ALERT*[3] reported a cluster of MRO observations of urine specimens that contained significant levels of *l*-methamphetamine. These specimens typically have significant levels of methamphetamine ranging from 4,000 ng/mL to over 15,000 ng/mL that are >80% *l*- (levo) methamphetamine. Many of the specimens are found to be 95-99% in the *l*- form. Episodic reports of these *l*-methamphetamine results continue and appear to be in geographical clusters. When contacted by the MRO, a few donors admitted to use of "illegal" methamphetamine. Reports from MROs continue to be made.

Results: Typically the well established criterion of reporting *d*- methamphetamine when it exceeds 20% of the total quantitative value in the derivitized chiral analysis is used. Thus illicit specimens that have more than 20% *l*-methamphetamine will be misinterpreted as "inhaler". For example, a urine with a concentration of 40,000 methamphetamine regardless of the relative percentage of *d* and or *l* isomer is not due to OTC nasal inhaler use, but misrepresents illicit methamphetamine with high levels of the *l*- isomer.

Conclusions: MROs need the legal regulatory guidance in the form of an upper level concentration of methamphetamine to rule out OTC use to support the correct verification of these presentations. The highest level of *l*-methamphetamine reported in the literature is less than 2,000 ng/mL. What is needed is a consensus on an upper level of total methamphetamine that to a high degree of certainty rules out OTC use of inhalers, similar to the way an upper level has been established for morphine to rule out poppy seed ingestion.

References:

1. Medical Review Officer Manual for Federal Agency Workplace Drug Testing Programs (effective October 1, 2010). http://www.workplace.samhsa.gov/Dtesting.html.
2. DEA Microgram Digest, published by the Drug Enforcement Administration Office of Forensic Sciences, Washington, DC 20537. Vol. XXXVI, No. 11, November 2003 and Vol. XXXVII, No. 4, April 2004
3. MROALERT, published by AAMRO –Vol. XIX, No. 4, May 2008

Keywords: Methamphetamine, Chiral Analysis, Workplace Drug Testing