S01

Subjective and Physiological Effects following Controlled Smoked, Vaporized, and Oral Cannabis Administrations to Frequent and Occasional Cannabis Smokers

Matthew N. Newmeyer*[1,2], Madeleine J. Swortwood[1], Osama A. Abulseoud[1], Marilyn A. Huestis[1,3]; [1]Chemistry and Drug Metabolism, IRP, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD, [2]Program in Toxicology, University of Maryland Baltimore, Baltimore, MD, [3]University of Maryland School of Medicine, Baltimore, MD

Background/Introduction: Cannabis is the most widely abused illicit drug worldwide. The active constituent Δ^9-tetrahydrocannabinol (THC) may produce desirable euphoric effects, but negative subjective and physiological changes may also occur. While cannabis is most commonly administered via smoking, vaporization and oral consumption are becoming increasingly popular. However, subjective and physiological effects following these alternative administrations compared to smoking are not thoroughly characterized.

Objective: Evaluate differences in subjective and physiological effects between smoked, vaporized, and oral cannabis in frequent and occasional cannabis smokers

Method: Eleven frequent (≥5x/week) and nine occasional (≥2x but <3x/week) cannabis smokers provided informed written consent for this National Institute on Drug Abuse Institutional Review Board-, FDA-, and DEA-approved study. The study was randomized, double-blind, and placebo-controlled with a crossover and double-dummy design. Over 4 dosing sessions, participants were administered one active (6.9% THC; 54mg) or placebo cannabis-containing brownie followed by either one active or placebo cigarette or one active or placebo vaporized cannabis dose. No more than one active dose was administered per session. Visual analog scales consisting of 100-mm lines anchored by "not at all" and "most ever" were presented before and up to 5h post-dose for 9 items or up to 72h (frequent smokers) or 48h (occasional smokers) for 6 items; participants' marked positions on the scales were converted to a percentage between 0-100. Heart rate (HR), systolic and diastolic blood pressure (SBP/DBP), and respiration rate (RR) were measured before and up to 3h post-dose. Data were analyzed via repeated-measures analysis of variance (ANOVA). Analyses were rerun with group data separated when significant group effects or interactions were present. Post-hoc tests with Bonferroni correction evaluated differences between administrations at each time point when significant interactions were observed. Finally, relationships between observed effects and matched blood THC concentrations were evaluated via linear mixed model analysis. Two-tailed $p<0.05$ indicated significance.

Result: Ratings (times differences were observed) for "Good Drug Effect" (0.25-3.5h), "High" (0.5-2.5h), "Stoned" (0.25-2.5h) and "Stimulated" (0.25-1.5h) following smoking and vaporization were significantly higher compared to placebo for all participants. Only frequent smokers' ratings for "Craving Marijuana" (0.25-3.5h) were significantly different from placebo following smoking and vaporization. Following oral cannabis administration, occasional smokers' ratings for "Good Drug Effect" (1.5-3.5h), "High" (0.5-3.5h), and "Stoned" (overall dose effect observed) were significantly higher and ratings for "Willing to Drive-Nonemergency" (1.5-3.5h), and "Willing to Drive-Emergency" (1.5-3.5h) were significantly lower than after placebo. For frequent smokers only, ratings for "Willing to Drive-Emergency" were significantly lower (overall dose effect) following oral dosing compared to placebo. Participants' HR changes from baseline at 0.5h following smoking (mean +11bpm) and vaporization (+10bpm) and changes from baseline at 1.5 (+9bpm) and 3.5h (+7bpm) following oral dosing were significantly larger than changes observed after placebo. Finally, significant relationships between blood THC concentrations and "Good Drug Effect", "High", "Stoned", "Stimulated", "Craving Marijuana", and HR following smoking and vaporization were observed.

Conclusion/Discussion: For the first time, differences in subjective and physiological effects following smoked, vaporized, and oral cannabis administrations were compared. Significant differences between smoked and vaporized cannabis were not present. Additionally, more group differences were observed following oral cannabis. These data help improve understanding of cannabis' effects following alternative administrations.

Keywords: Cannabis, Pharmacodynamics, Blood

S02

Distribution of QT-Prolonging Drugs and Metabolites Between Cardiac Tissue and Femoral Blood in a Psychiatric Population

Christian Reuss Mikkelsen[1]*, Jakob Ross Jornil[1], Ljubica Vukelic Andersen[1], Jytte Banner[2], Jørgen Bo Hasselstrøm[1]; [1]Department of Forensic Medicine, Aarhus University, Denmark, [2]Department of Forensic Medicine, Copenhagen University, Denmark

Background/Introduction: The Danish forensic autopsy-based prospective study, named SURVIVE, aims at elucidating the causes of the increased mortality and morbidity among mentally ill patients. Mentally ill patients are a heavily medicated patient group with high risk of early and sudden death. Many antipsychotics, antidepressants, and some substances of abuse consumed by mentally ill patients have been found to prolong the QT interval of the ECG. This can lead to the ventricular arrhythmia condition Torsades de Pointes, which in turn can lead to sudden cardiac death. In forensic postmortem toxicology, the blood concentrations are usually used for estimating a possible death by cardiac arrhythmia; however, animal studies and postmortem case studies have shown that some QT-prolonging drugs (QTDs) can be found in higher concentration in cardiac tissue when compared to peripheral blood.

Objective: The aim of this project is to characterize the distribution of seven frequently used QTDs and their metabolites in cardiac tissue and femoral blood, and to investigate if the cardiac tissue concentration could be a tool for estimating cardiac arrhythmia as a potential cause of death.

Method: Inclusion for SURVIVE was known or suspected mental illness and/or use of psychoactive medication at time of autopsy. Among the 500 cases included in SURVIVE, cases with at least one of QTD reported was selected for this project. A sample preparation method was developed and validated for the purpose of this project. After cardiac tissue homogenization, the cardiac homogenate and blood followed the same protein precipitation and filtration method. Deuterated internal standards were used. The extracts were analyzed on a Waters ACQUITY UPLC with a Waters Xevo TQ-S. Sex, age, BMI, manner and cause of death and time of last seen alive, declared death and autopsy as well as toxicology results were obtained from police, toxicology and autopsy reports for the descriptive analysis. Based on the toxicological evaluation, each compound result was classified A, B and C according to intoxication status.

Result: Of the 500 SURVIVE cases, 211 cases were included in this project; 121 males and 90 females ranging from 18 to 94 years old. Each compound was found in between 8 and 90 cases each. The compound concentrations in whole blood and cardiac tissue were found to be statistically correlated ($p<0.05$) with 12 compounds very strong correlated ($R^2>0.8$), one strong correlated ($R^2=0.76$) and one moderate correlated ($R^2= 0.62$). Across all 14 compounds the heart-to-blood ratio (HB-ratio) ranged from 0.093 to 43 and the median HB-ratio ranged from 1.6 to 14. Each compound showed a high variance in HB-ratio between cases which was addressed statistically. Only age showed to be statistically significant in explaining the high HB-ratio variance of each compound. No difference in HB-ratios was detected due to extended postmortem interval.

Conclusion/Discussion: In this project, we present the distribution of frequent used QTDs in the SURVIVE population between cardiac tissue and femoral blood.

Keywords: QT-Prolonging Drugs, Heart-to-Blood Concentration Ratio, Forensic Toxicology

S03

ELISA with Reflex to LC-MS/MS for Detection and Quantitation of Buprenorphine and Norbuprenorphine in Serum or Plasma

Brandy M. Hill*[1], Stephanie J. Marin[2], Gwendolyn A. McMillin[1,3]; [1]ARUP Laboratories, [2]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [3]University of Utah Department of Pathology, Salt Lake City, UT

Background/Introduction: Buprenorphine is a partial opioid agonist-antagonist used to treat chronic pain and opioid addiction. Routine monitoring of buprenorphine compliance is recommended. Testing buprenorphine in blood avoids concerns about specimen adulteration that are common in high risk populations tested with urine. Here an enzyme-linked immunosorbent assay (ELISA) screen and confirmation assay performed by LC-MS/MS were validated to support clinical buprenorphine testing with serum/plasma. Steady state plasma concentrations of buprenorphine and norbuprenorphine in patients on a maintenance dose of buprenorphine (4-8 mg/day) can be as low as 0.8 ng/mL for buprenorphine and 1.1 ng/mL for norbuprenorphine (1). Glucuronide metabolites of buprenorphine and norbuprenorphine are present in serum and plasma. To maximize detection of buprenorphine and norbuprenorphine, enzyme hydrolysis and a cutoff of 1 ng/mL were implemented for the LC-MS/MS assay.

Objective: To evaluate compliance for buprenorphine with the most sensitive assay possible.

Method: An ELISA kit (part #236-0096, Immunalysis, Pomona, CA) was validated using an aliquot (10uL) of diluted serum/plasma. Qualitative accuracy and precision were determined with spiked samples. A quantitative LC-MS/MS confirmation assay was also validated. Pre-analytical enzymatic hydrolysis and solid phase extraction were performed prior to analysis by LC-MS/MS.47 serum/plasma specimens submitted for buprenorphine quantitation were analyzed and results compared to a previously validated in-house LC-MS/MS method that did not use hydrolysis and had a lower limit of quantitation (LLOQ) of 2 ng/mL. 20 residual patient specimens (10 positive and 10 negative) previously analyzed by Drug Detection Panel by High-Resolution Time-of-Flight Mass Spectrometry Serum or Plasma, that included buprenorphine (5ng/mL) and norbuprenorphine (10 ng/mL) were also evaluated. Specimens were de-identified following a University of Utah Institutional Review Board (IRB) protocol.

Result: The cutoff for the ELISA assay was 5 ng/mL. Norbuprenorphine demonstrates 120% cross reactivity and buprenorphine 100% at this concentration. Evaluating spiked samples, 43% of samples at the cutoff (n=14) screened positive; all samples spiked at 50% (n=14) and 70% (n=28) of the cutoff screened negative; all samples spiked at 130% (n=14) and 150% (n=28) of the cutoff screened positive. Average imprecision around the 5ng/mL cutoff was 13% over 7 days. The analytical measurement range for the LC-MS/MS assay was 1-100 ng/mL. Accuracy of calibrators, QC, and spiked samples were within ±10% of expected values for buprenorphine and norbuprenorphine. Average between run imprecision (%CV) was within ±10%. Recovery ranged from 70-112% for both compounds. 38 of the 47 positive patient specimens from the previously validated LC-MS/MS assay confirmed positive for buprenorphine (81%), and 34 of the specimens confirmed positive for norbuprenorphine (72%). 14 specimens that failed to confirm using the previously validated method were positive by the new method. This resulted from employing enzymatic hydrolysis and lowering the LLOQ to 1 ng/mL. Nine of the 10 TOF positive specimens were positive, and the 10 TOF negative specimens were negative using the new LC-MS/MS method.

Conclusion/Discussion: Enzyme hydrolysis of serum/plasma specimens improved detection for clinical monitoring of buprenorphine. An LLOQ of 1 ng/mL is appropriate for analysis of specimens from patients prescribed buprenorphine. The Immunalysis ELISA for serum/plasma buprenorphine was accurate and precise at 5ng/mL.

Keywords: Buprenorphine, LC/MS-MS, ELISA

S04

The Presence of Parent Cocaine in the Absence of Benzoylecgonine in Urine Specimens of Healthcare Patients

Kate Claussen[1,2], **Brandi Puet*[1,3],** Anne DePriest[1,3], Rebecca Heltsley[1], Timothy Robert[1], David L. Black[1,4]; [1]Aegis Science Corporation, Nashville, TN, [2]Belmont University, College of Pharmacy, Nashville, TN, [3]University of Tennessee Health Science Center, College of Pharmacy, Memphis, TN, [4]Vanderbilt University, Department of Pathology, Immunology and Microbiology, Nashville, TN

Background/Introduction: Cocaine is currently abused by over 1.5 million individuals and is the number one illicit drug responsible for emergency department visits in the United States. With clinical drug testing, analysis for parent cocaine (COC) is not a common practice due to rapid hydrolysis and short detection period. Benzoylecgonine (BZE), a major metabolite, may be detected in urine for up to several days post cocaine use.

Objective: To characterize cocaine and metabolite disposition in urine of healthcare patients with a focus on COC-only results**;** to consider patient factors that may alter metabolic disposition.

Method: A database was created from all specimens submitted to Aegis by pain management and addiction treatment providers from January to July 2015 that were positive for cocaine use, as determined by the presence of COC and/or BZE above the limit of quantitation (LOQ, 50 ng/mL). All specimens were analyzed by liquid chromatography-tandem mass spectrometry (LC–MS–MS) analyses.

Result: A total of 7,832 urine specimens from 6,151 patients were positive for cocaine. Specimens originated from 749 clinics in 39 states. Of these specimens, 96.8% and 26% were positive for BZE and COC, respectively. A total of 74% of specimens yielded detectable BZE only. Of interest, 3.2% of positive specimens were positive for COC in the absence of BZE. Of 203 patients with COC-only results, 30 patients had multiple specimens with this pattern. One patient provided 5 specimens with COC-only results. Forty-eight patients with COC-only results provided additional specimens with BZE present.

Conclusion/Discussion: Inter-patient variability in metabolic enzyme activity has been reported and can cause unexpected excretion patterns. However, the presence of parent cocaine without metabolite has not been previously reported or elucidated. Factors such as enzyme deficiencies and drug interactions may lead to variability in excretion patterns. This report highlights the potential utility of testing for COC in addition to BZE in the clinical setting.

Keywords: Cocaine, Benzoylecgonine, Urine

S05

Incidence of Mitragynine Abuse and Observed Concentrations in Court Ordered Drug Testing Cases

Lister M. Macharia*, Brent Dawson, Sumandeep Rana; Redwood Toxicology Laboratory, Santa Rosa, CA

Background/Introduction: *Mitragyna speciosa,* Korth (kratom, Biak Biak) is a tropical tree native to Southeast Asia, mainly Thailand, Malaysia, Indonesia, and Myanmar. Traditionally, fresh or dried leaves were chewed or made into tea and used as a stimulant to alleviate pain and increase energy. It has also been used recreationally as an opiate substitute, to aid opioid withdrawal symptoms, and in the management of coughs and diarrhea. Mitragynine has gained popularity in recent years as a "natural legal high" that's readily available in head shops and online in various formats. While it is not currently scheduled in the U.S., kratom is classified as having no legitimate medicinal use and is on the DEA list of drugs to watch.

The laboratory sought to validate a simple and fast method that can be reliably used to determine mitragynine in urine. The fully validated LC/MS/MS method has since been used to analyze over 9,000 urine specimens from court ordered drug testing programs. The results from the validation and clinical specimens will be discussed.

Objective: To develop and validate a simple protein precipitation and fast LC/MS/MS acquisition method for determination of mitragynine in urine and to evaluate the concentrations and overall positivity of samples analyzed.

Method: Method validation studies included accuracy, imprecision, LOD/LLOQ, ULOL,carryover, matrix effects, stability, and interference from over 100 related and non-related drugs. Calibration solutions were spiked using mitragynine drug standard and its deuterated analogue (Cerilliant Corporation). Specimen preparation involved protein precipitation of 100 µL of sample using 100 µL of acetonitrile spiked with mitragynine-D3 internal standard at 100 ng/mL. Analysis was performed on a Prominence Liquid chromatograph (Shimadzu) coupled to a 4000 QTrap mass spectrometer (SCIEX) by injecting 20 µL on to an Ultra biphenyl, 5 µm x 50 mm x 2.1 mm column. Mobile phases constituted 0.1% formic acid with 2 mM ammonium formate and 0.1% formic acid with 2 mM ammonium formate in acetonitrile. At a flow rate of 0.7 mL/minute, the gradient started at 20% organic, increasing to 90% in 1.1 minutes before returning to original conditions in a total run time of 3.3 minutes. Method was applied to over 9,000 authentic urine specimens with a cut-off of 1 ng/mL.

Result: The LLOD and LOQ were 1 ng/mL and the ULOL was 2,500 ng/mL, giving an $R \geq 0.99$. Inter and Intra-day imprecision was below 3% with accuracies between 90-110%. No carry over was observed for concentrations up to 2,500 ng/mL. The %CV of the internal standard normalized matrix factors was ≤5%, well within the <15% SWGTOX acceptable criteria. No interference was observed from any of the spiked drugs (5,000 ng/mL) in negative or LLOQ specimens. Prepared specimens were stable in the instrument autosampler (15^0C) for 48 hrs.

Conclusion/Discussion: Analysis of mitragynine in urine was successfully implemented in our laboratory using simple protein precipitation and fast LC/MS/MS acquisition**.** It can be reliably detected and quantified over a robust linear range from 1 – 2,500 ng/mL. Actual concentrations in urine vary widely across the linear range, with 23% of positive samples having concentrations below 10 ng/mL and 22 % above 1,000 ng/mL. This demonstrates a need for a wide linear range for the assay and is also useful in setting cutoff concentrations.

Keywords: Kratom, Mitragynine, Urine

S06

Method Validation for the Quantification of Buprenorphine in Blood and Urine Using Two-Dimensional Gas-Chromatography-Mass Spectrometry

Rachel Callaway*, Christopher Rosales, Protiti Sarker, Rong-Jen Hwang; Toxicology Bureau – Scientific Laboratory Division – New Mexico Department of Health, Albuquerque, NM

Background/Introduction: Buprenorphine is a μ-Opioid receptor partial agonist used for pain management and opioid withdrawal. Since its introduction to the US market in the early 2000s as an alternative to methadone, its use has been steadily increasing. Buprenorphine has analgesic properties up to 40X that of morphine which contribute to its potential for abuse and a need for a reliable quantitation method in forensic labs. A low therapeutic range makes it difficult to detect using conventional GC/MS methods, so a method was developed and validated using two dimensional gas chromatography mass spectrometry (2D-GC/MS) to resolve matrix interference and improve sensitivity.

Objective: A solid phase extraction (SPE) followed by 2D-GC/MS method was developed and validated for quantification of buprenorphine in DUID and postmortem analysis in whole blood and confirmation in urine. Use of a Deans switch allowed resolution of the drug of interest away from the matrix onto a secondary GC column while cryofocusing enhanced the signal. This lab does not routinely quantify drugs in urine so a hydrolysis procedure was not necessary. The method was validated following SWGTOX guidelines.

Method: Buprenorphine-D4 internal standard was added to samples, and proteins were precipitated by addition of 2.0 mL cold acetonitrile with vigorous vortexing. Samples were then centrifuged and the supernatant transferred to a clean tube and acidified by addition of 0.1 M HCl. This was then applied to SPEWare Cerex Clin II SPE columns. Four wash steps consisting of deionized water, 0.1 M HCl, methanol and ethyl acetate were performed before drying the columns and eluting the analyte with 78:20:2 dichloromethane: isopropanol: ammonium hydroxide. After evaporation to dryness, samples were derivatized by BSTFA with 1% TCMS. The trimethylsilyl derivatives were then injected onto an Agilent 7890B/5977A 2D-GCMS equipped with a J&W Scientific DB-1 MS (15 m, 0.25 mm I.D., 25 μm) primary column and a DB-17 MS (15 m, 0.25 mm I.D. 0.25 μm) secondary column. A timed cut including the buprenorphine and buprenorphine-D4 was diverted from the primary column and cryofocused onto the secondary column which gave the sample a second degree of resolution before passing into the mass spectrometer. Samples were analyzed using electron impact-select ion monitoring (SIM) using three ions for both the analyte and the internal standard. Validation was performed using SWGTOX guidelines for calibration model fits, accuracy and precision, sensitivity measured by the limit of detection (LOD) and limit of quantitation (LOQ), carryover, interference, dilution integrity and stability.

Result: Buprenorphine was found to fit to two curves: a low curve spanning 1.5 – 10 ng/mL and a high curve from 10 – 45 ng/mL with a fit of 1/x for both curves and average R^2 values of 0.9986 and 0.9994. The LOQ was determined to be 1.5 ng/mL with a calculated LOD of < 0.5 ng/mL. Percent accuracy at three concentrations was excellent, ranging from 99.5% to 100%, with an inter-run precision %CV ranging from 0.5% to 4.4%. The largest intra-run %CV was used to determine precision acceptability which was 2.4% at the lowest and 3.6% at the highest, well below the allowable ± 20%. No significant carryover or interference from matrix effects or drugs of abuse were noted. Dilutions of 1:2, 1:4 and 1:10 were quantitated with accuracies ranging from 89.6% to 94.2%, inter-run %CV of 5.7%-7.3% and largest intra-run %CVs of 4.3% - 8.9%. Extracted samples were stable over five days.

Conclusion/Discussion: Currently there is one other published 2D-GC/MS method for quantification of buprenorphine validated for postmortem blood using MSTFA as a derivatizing agent. This laboratory contributes a second differing by matrix, instrumentation and derivatizing reagent for routine analysis in DUID and postmortem cases. The limitation of this method did not allow for identification of norbuprenorphine. Case samples where buprenorphine was suspected were re-extracted and the drug was confirmed and quantitated with this new method.

Keywords: Buprenorphine, 2D-GC/MS, Validation

S07

Quantitation of Cocaine and Metabolites, Barbiturates, and Phencyclidine in Meconium by LC-MS/MS

Triniti L. Scroggin*[2], Natalie N. Rasmussen[2], Gwendolyn A. McMillin[2,3]; [1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]ARUP Laboratories, Salt Lake City, UT, [3]University of Utah Department of Pathology, Salt Lake City, UT

Background/Introduction: The use of drugs during pregnancy can lead to varying complications and potentially permanent damage to the fetus, thus early detection of drug exposure is critical. Meconium, unlike urine, can reflect drug abuse during the third trimester of a full term birth (1). The wide range of concentrations of drugs and metabolites observed in meconium make it challenging to create multidrug assays and to interpret results (2). Currently, meconium testing for phencyclidine, cocaine and metabolites, and barbiturates is completed in our laboratory with three GC-MS assays. A polarity-switching LC-MS/MS method was developed to quantitate phencyclidine, three cocaine metabolites, and two barbiturates in a single assay.

Objective: To develop a single polarity-switching LC-MS/MS assay that uses a single sample preparation to combine three separate GC-MS assays.

Method: Previously screened negative meconium specimens were used to prepare calibrators and controls. De-identified residual patient specimen, calibrators and controls (25% LLOQ and 75% ULOQ) were weighed (0.25g ±0.01), internal standard was added, homogenized in buffer, and centrifuged at 0ºC. Congealed soluble lipids were removed, and the supernatants were extracted using Phenomenex XL-C 60mg SPE columns (Phenomenex, CA). Once loaded, the adsorbents were washed with 1mL of nanopure water, 0.1M acetic acid, and 75:25 methanol:water; the sorbents were dried for 8 minutes at 50 psi. Barbiturates (butalbital and phenobarbital) were eluted into autosampler vials using 1mL of 50:50 hexane:ethyl acetate. Columns were washed with 1mL of methanol; the sorbents were dried for 2 minutes at 50 psi and the remaining drugs were eluted into the vials with the first elution with 68:30:2 ethyl acetate:methanol:ammonium hydroxide. The combined eluent were dried under nitrogen at 25ºC for 20 minutes and the residues were reconstituted with 600µL of water. Samples were analyzed using an Agilent 1200 HPLC (Santa Clara, CA, USA) coupled to a Sciex AB4000 tandem mass spectrometer (AB Sciex,Foster City, CA). Chromatographic separation was performed using a Phenomenex Kinetex 5µm Phenyl-Hexyl 50x3.0 mm column with a flow rate of 500µL/min. Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in 50:50 methanol:acetonitrile. Peaks of barbiturates were sufficiently resolved from the remaining targeted drugs to allow a single polarity switch from positive to negative mode.

Result: Patient specimen previously analyzed using the validated GC-MS methods (n=3) were used for method comparison see Table 1. Forty-four patient specimens were tested; some analytes were spiked into positive patient specimens to account for the low positivity rate in clinical samples. Accuracy and precision for controls (QC) was analyzed in triplicate over 4 days, see table 2 (n=12 per compound). The analytical measurement range was 10-1000 ng/g for phencyclidine, 20-2000 ng/g for cocaine and metabolites, and 50-5000 ng/g for barbiturates.

Table 1: Patient Correlation and Spike Sample Results

Compound	Average Accurac
Phencyclidine (n=21)	84.4%
Cocaine (n=23)	84.9%
Cocaethylene (n=12)	82.9%
Benzoylecgonine (n=33)	83.4%
M-Hydroxy-Benzolecgonine	84.9%
Butalbital (n=24)	84.4%
Phenobarbital (n=12)	89.4%

Table 2: QC Results

Compound	Accuracy		%CV	
	Low	High	Low	High
Phencyclidine	102%	104%	11%	1%
Cocaine	85%	102	1%	1%
Cocaethylene	92%	89 %	7%	11%
Benzoylecgonine	85%	92%	1%	1%
m-OH-	89%	94%	10%	3%
Butalbital	102%	95%	3%	1%
Phenobarbital	104%	98%	6%	1%

Conclusion/Discussion: This combined method was shown to have adequate accuracy and precision for the measurement of phencyclidine, cocaine and three metabolites and two barbiturates. The method allows accurate quantitation of targeted drugs in a complex matrix while significantly reducing sample preparation and analysis time.

Keywords: Meconium, Polarity-Switch, LC-MS/MS

S08

Brain on Alcohol: Method for Alcohol Analysis in Brain Tissue by Headspace Gas Chromatography with Flame Ionization Detector (HS-GC-FID)

Hao-Jung Chun, **Carl E. Wolf*,** Justin L. Poklis, Alphonse Poklis; Department of Forensic Science, Pathology, and Pharmacology & Toxicology at Virginia Commonwealth University, Richmond, VA

Background/Introduction: Ethanol is the most widely used and abused drug. While blood is the preferred specimen for analysis, tissue specimens such as brain can serve as alternative specimens for alcohol analysis in postmortem cases where blood is unavailable or potentially contaminated. Where blood is easily lost due to antemortem trauma, the brain may be preserved due to its encasement in its protective skull. The isolated location and lack of glucose storage also make the brain an attractive specimen for analysis as it is less susceptible to postmortem alcohol diffusion, such as from the stomach to heart blood, or postmortem alcohol formation. There are concerns over differences in alcohol concentration in postmortem blood collected from various body sites, the regional distribution of alcohol in the brain does not differ significantly. As a highly vascularized tissue with a rich blood supply, the brain displays rapid alcohol equilibrium with blood and thus may be a good indicator of antemortem blood alcohol content. Though the value of brain as an alternative specimen for postmortem alcohol analysis cannot be overstated, unlike other alternative specimens, there have been few studies performed to analyze alcohol concentrations in brain.

Objective: Validate a Headspace-Gas Chromatographic Flame Ionization (HS-GC/FID) method for the detection and quantification of ethanol and other volatiles including acetone, isopropanol, methanol, and n-propanol, in brain tissue specimens using t-butanol as the internal standard (ISTD) and following the Scientific Working Group for Forensic Toxicology (SWGTOX) recommended standard practices for method validation in forensic toxicology.

Method: Samples were prepared by adding 0.9 mL of 1580 mg/kg t-butanol (ISTD) in water to 400 µL of a 1:3 (tissue:water) homogenate in a 22 mL auto-sampler vial. Vials were then capped. The analysis was performed on a Varian 3900 GC with an FID detector (Varian Associates, Inc., Walnut Creek, CA) equipped with a Rtx®–BAC2 column, 30 m 0.53 mm ID 2.0 µm (Restek Corp., Bellefonte, PA). The GC oven and detector temperatures were 40 and 235°C, respectively. The carrier gas was helium (16.8 mL/min) and the detector gas was helium makeup (25 mL/min), hydrogen (30 mL/min), and air (300 mL/min). Under these conditions, the retention times of methanol, ethanol, acetone, isopropanol, ISTD, and n-propanol were 1.66, 2.16, 2.31, 2.53, 2.80, and 4.05 min, respectively. The auto-sampler was a Tekmar 7000 headspace auto-sampler with a Hydroguard MXT® 2 m 0.53 mm ID (Restek Corp.) sample loop. The platen temperature was 80°C with 0.1 min platen equilibrium time, 3.5 min sample equilibration time, 0.2 min mixing time, and 0.5 min stabilization time. The sample loop temperature was 160°C, fill time was 0.2 min, equilibrium time was 0.2 min, and injection time was 0.3 min.

Result: The presented HS-GC-FID method was validated using the SWGTOX recommended standard practices. The limit of detection and limit of quantification were administratively set at 100 mg/kg. The upper limit of quantification was 5000 mg/kg. The within-run and between-run bias of the method was ≤ 10% and the precision was ≤ 4% coefficient of variation (CV) for all controls. No carryover or endogenous interferences were detected. The assay was determined to be sensitive and selective. The volatiles were determined to be stable in the QC specimens.

Conclusion/Discussion: The presented HS-GC-FID method for the detection and quantification of ethanol, acetone, isopropanol, methanol, and n-propanol in brain tissue was sensitive, selective, reliable and robust in postmortem alcohol analysis in brain tissue specimens. A linear calibration model was found to be appropriate for all analytes and no significant matrix effect was observed when compared to aqueous calibration.

Keywords: Ethanol, Brain, Headspace

S09

Synthetic Cannabinoid Screening and Confirmation in Whole Blood Using an Agilent 6430 QQQ and Agilent 6550 QTOF

Jason S. Hudson*, Rebekah Boswell, Dina Swanson, Curt E. Harper; Alabama Department of Forensic Sciences, Hoover, AL

Background/Introduction: In recent years, synthetic cannabinoids have received a large amount of attention in forensic toxicology due to their effects on human performance and behavior, as well as implications for cause of death analysis. Development of analytical methods for the screening and confirmation of synthetic cannabinoids in whole blood has been challenging due to low biological concentrations and the rapidly increasing number of compounds. Triple quadrupole (QQQ) and quadrupole time of flight (QTOF) mass spectrometers are becoming more common in forensic laboratories due to their high sensitivity and mass resolution capabilities. The work presented here demonstrates a screening and confirmation methodology by mass spectrometry that exploits the ability of these mass spectrometers to screen with high sensitivity and confirm their presence with MRM transitions and accurate mass.

Objective: Our aim is to develop a qualitative method to screen and confirm ten synthetic cannabinoids in blood using an Agilent 6430 QQQ and an Agilent 6550 QTOF.

Method: We evaluated the following synthetic cannabinoids: UR-144, XLR-11, AB-PINACA, JWH-18, AB-CHIMNACA, PB-22, AB-FUBINACA, JWH-210, MAB-CHIMNACA, and ADB-FUBINACA. We compared four extraction procedures (one protein precipitation, one liquid-liquid extraction, and one solid phase extraction) and determined the sensitivity (LOD) for the instruments. The protein precipitation extractions utilized acetonitrile and methanol as organic solvents, the solid phase columns were Phenomenex Strata-X B, and the liquid liquid extraction was conducted by adding water and acetic acid, then extracting with 9:1 hexane/ethyl acetate. The extraction procedure that had the best combination of sensitivity and simplicity was selected for use. After extraction, the samples were chromatographically separated by an HPLC (QQQ) and UHPLC (QTOF) system using an Agilent Poroshell EC-C18 column with ammonium formate/0.1% formic acid and methanol/0.1% formic acid as the mobile phases. The modes of analysis for detection were targeted MRM for the QQQ, and MS-Only and All-ions modes for the QTOF. Authentic positive synthetic cannabinoid cases were evaluated for proof of principle.

Result: The ACN protein precipitation crash was selected for use due to its simplicity and the target compounds high degree of sensitivity for the QQQ and QTOF instruments. We monitored two MRM transitions for the QQQ experiment with acceptance criteria being retention time, S/N greater than three, and MRM transition ratios within +/-20% of a control. For the QQQ, all targets with the exception of AB-FUBINACA, had a LOD of 0.05ng/mL. Data handling and acceptance criteria for the QTOF in MS-Only mode relied on accurate mass extraction and retention time from an in house database. In addition, a scoring algorithm for determining positive results was used that included weighted scores for accurate mass, isotopic abundances/spacing, and retention time. The All-ions mode was also utilized for the QTOF experiments that provided fragment confirmation with a requirement of the presence of three specific ions from a spectral library. For the QTOF, the LODs were highly variable for the ten targets, with AB-PINACA not being detected. Utilizing the selected extraction and instrumental parameters, authentic samples were subjected to both QQQ and QTOF analysis for screening and confirmation. The results for the authentic samples were consistent with the results from the reference laboratories used for confirmation.

	Limits of Detection (ng/mL)							
	ACN Crash		*MeOH Crash*		*SPE*		*LLE*	
Target	**QTOF**	**QQQ**	**QTOF**	**QQQ**	**QTOF**	**QQQ**	**QTOF**	**QQQ**
UR-144	0.05	0.05	0.25	0.25	0.25	0.05	0.50	0.05
XLR-11	0.10	0.05	0.50	0.05	0.25	0.05	0.25	0.05
AB-PINACA	ND	0.05	ND	0.05	ND	0.05	ND	0.05
JWH-18	0.05	0.05	0.25	0.05	0.25	0.05	0.25	0.05
AB-CHIMNACA	5.0	0.05	100	0.05	ND	0.05	ND	0.05
PB-22	0.10	0.05	0.50	0.05	0.50	0.05	0.25	0.05
AB-FUBINACA	0.25	0.25	5.0	0.25	10	0.10	5.0	0.05
JWH-210	0.50	0.05	5.0	0.05	0.25	0.05	0.25	0.05
MAB-CHIMNACA	0.50	0.05	5.0	0.05	10	0.10	5.0	0.05
ADB-FUBINACA	0.05	0.05	100	0.05	0.25	0.05	0.10	0.05

Conclusion/Discussion: We offer a unique synthetic cannabinoid screen and confirmation method in blood using the combination of QQQ and QTOF technologies. Our data shows better sensitivity was achieved by targeted MRM analysis with the QQQ when compared to the QTOF. However, the use of both for screening and confirmation allows for the exploitation of the ruggedness and sensitivity of the QQQ, and the ability to identify drugs by accurate mass and retroactively screen unknowns by the QTOF.

Keywords: QTOF, LC/MS/MS, Synthetic Cannabinoids

S10

Validation of an LC-MS/MS Method for Analysis of U-47700, U-50488 and Furanyl Fentanyl, and its Application in Postmortem Casework

Amanda LA Mohr*[1], Melissa Friscia[1], Donna M Papsun[2], Sherri Kacinko[2], Barry K. Logan[2]; [1]The Center for Forensic Science Research and Education, Willow Grove, PA, [2]NMS Labs, Willow Grove, PA

Background/Introduction: Following waves of synthetic cannabinoids and synthetic cathinone derivatives, the illicit drug market has begun to see increased use of illicit synthetic opioids including fentanyl and its derivatives, and other chemically unrelated compounds, AH7921, and MT45. Among the most frequently encountered compounds in postmortem casework have been furanyl fentanyl (*N*-(1-(2-phenylethyl)-4-piperidinyl)-N-phenylfuran-2-carboxamide, Fu-F), and U-47700 (*trans*-3,4-dichloro-*N*-(2-(dimethylamino)cyclohexyl)-N-methylbenzamide). Both drugs have been reported to be present in the heroin supply and to be gaining popularity among recreational opioid users. Both drugs were developed by pharmaceutical companies in the 1980's as candidates for development as potential analgesic therapeutic agents. Furanyl fentanyl is believed to have potency similar to fentanyl. U-47700 is selective for the μ-opioid receptor, and in animal models demonstrated approximately 7.5 times the potency of morphine. A related compound, U-50488 (*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide), is an agonist at the κ-opioid receptor, but without any reported μ-opioid receptor antagonistic effects. We describe the development and validation of an analytical method for these compounds and its application in the analysis of suspected narcotic analgesic related deaths.

Objective: The purpose of this presentation is to describe the development and validation of a method for the analysis of furanyl fentanyl, U-47700 and U-50488 in blood, urine and other tissues from suspected opioid related deaths.

Method: Analysis was performed using an Agilent® 1100 Series Liquid Chromatograph coupled to an Agilent® 6430 Tandem Mass Spectrometer. A fit-for-purpose validation was performed over the course of three days and evaluated for limit of detection and quantitation, linear dynamic range, robustness, accuracy, bias and precision. Samples (500 μL) were extracted using a solid phase extraction procedure. Samples were pretreated with phosphate buffer, mixed and centrifuged prior to being applied to the column. The columns were conditioned with methanol, deionized water and phosphate buffer. After the application of the sample, columns were washed with deionized water, acetic acid and methanol. The samples were eluted with ethyl acetate/acetonitrile/ammonium hydroxide (78:20:2). The organic phase was evaporated to dryness and reconstituted in 60:40 0.1% formic acid in water and 0.1% formic acid in methanol. Blood samples were processed against the calibration curve. All other specimens were processed via standard addition with blanks and two spikes. The calibration range was established from 1-500 ng/mL for U-47700 and U-50488 and from 1-100 ng/mL for furanyl fentanyl. The limit of detection was established at 0.5 ng/mL for all compounds.

Result: Samples from twenty-two cases involving deaths occurring between December 2015 and March 2016, and suspected of involving furanyl fentanyl and/or U-47700 were submitted for analysis. Of the three compounds in the scope, 11 cases were positive for U-47700 only, four cases were positive for furanyl fentanyl only and four cases contained both compounds. U-50488 was not detected in any of the cases. The mean and median (N=10) concentrations for furanyl fentanyl in whole blood were 16 ng/mL (±19) and 6.5 ng/mL and were all detected within a range of 1.9-57 ng/mL. For U-47700 the mean and median concentrations (N=16) were 253 ng/mL (±150) and 247 ng/mL with range of 17-490 ng/mL. Among the above cases, 95% were male, 5% female, and the mean age was 28.

Conclusion/Discussion: Within toxic deaths, opiates are among the most frequently encountered drugs. In the cases described above, most were suspected of being opioid-related deaths. Most deaths were attributed in whole or in part to the use of the synthetic opioids U-47700, furanyl fentanyl or a combination of both drugs. The analytical results in these cases establish the first reported post-mortem blood concentrations for U-47700 and furanyl fentanyl.

Keywords: U-47700, Furanyl Fentanyl, U-50488

S11
Synthetic Opioids in Fatalities – a UK Perspective

Simon Elliott*; ROAR Forensics (Alere), Worcestershire, UK

Introduction/Objective: In recent years, synthetic opioids have become a new entrant in the "designer drug" arena. Routine toxicological analysis by HPLC-DAD, LC-MS and QTOF-MS has allowed their detection and identification in casework. The objective of this study was to audit the number and nature of occurrences in post-mortem situations.

Methods/Results: Between February 2013 and January 2016, synthetic opioids have been detected in 11 fatality investigations by the laboratory and were the cause of death or were likely to have contributed to the cause of death in 82% of cases (9 fatalities). Where information has been available, they appear to have been sourced (usually as powder) via "research chemical" websites or related retailers. The first synthetic opioid encountered was AH-7921 and was subsequently found in 5 deaths until December 2014 with post-mortem femoral blood concentrations of 0.05, 0.35, 0.58, 0.84 and 4.46 mg/L. Other drugs and/or alcohol were detected in all of the cases but only contributed or provided an alternative cause of death in 2 of the cases (associated with femoral blood concentrations of 0.05 and 0.35 mg/L). Following AH-7921's control under the UK Misuse of Drugs Act 1971 in January 2015, acetylfentanyl was identified between February and October 2015 in 4 fatalities, being the cause of death or contributing to the cause of death in all cases. Post-mortem femoral blood concentrations of 0.12, 0.3, 1.35 and 1.40 mg/L were measured and again other drugs and/or alcohol were present in all cases but of less toxicological significance than acetylfentanyl. In July 2015 a further fentanyl derivative (para-fluorobutyrfentanyl) was detected along with low levels of 3-fluorophenmetrazine (3-FPM), methiopropamine (MPA), clomipramine, and alcohol (67 mg/dL). However the most significant finding was the presence of O-desmethyltramadol (ODT), another synthetic opioid and active metabolite of the prescription analgesic tramadol. ODT powder itself was found at the scene and a fatally toxic femoral blood concentration of 7.53 mg/L was measured. No other fentanyl derivatives have been detected in post-mortem casework since but in January 2016, U-47700 (a more potent structural derivative of AH-7921) was detected in a fatality. The circumstances involved the snorting of a powder (determined to be U-47700) by an individual suspected of using alleged "legal highs". U-47700 was present in the post-mortem femoral blood at a concentration of 1.46 mg/L, higher than many of the AH-7921 cases. Whilst other drugs were present in the urine only or at low levels in the blood (cannabis, mexedrone, amphetamine, ketamine, naproxen, quetiapine), U-47700 presented a greater toxicological significance with no apparent competing cause of death. During the investigation an analytical methodology was developed to differentiate between U-47700 and AH-7921. These two compounds share the same empirical formula and hence identical molecular weight, which is significant where only accurate mass spectral analysis (without fragmentation) is used for drug detection.

Conclusion: Due to their pharmacological nature, synthetic opioids present a significant toxicological risk to users, many of which are poly-drug users. Laboratories should therefore be aware of their existence and develop appropriate and selective methods for their detection.

Keywords: Synthetic Opioids, Postmortem, Research Chemicals

S12
Molly, Daffy, and Kiddy-Coke Walk into a Rave: A Case of Polysubstance Overdose

Sara K. Dempsey*, Carl E. Wolf, Justin L. Poklis, Alphonse Poklis; Departments of, Pharmacology & Toxicology, and Pathology, Virginia Commonwealth University, Richmond, VA

Background/Introduction: It is well known that methylphenidate, a central nervous stimulant used to treat ADHD and narcolepsy, is commonly used and abused by adolescents and adults due to its stimulant effects. Methylphenidate blocks the reuptake of dopamine and norepinephrine, leading to an increase of these neurotransmitters primarily in the prefrontal cortex. Though modafinil, a wake-promoting agent used to treat narcolepsy, is not a commonly abused drug, studies have shown that it has potential for abuse and dependence. Modafinil acts as an atypical dopamine reuptake inhibitor to increase dopamine levels primarily in the nucleus accumbens. It also increases serotonin and norepinephrine in other rewarding areas of the brain. 4-Fluoroamphetamine ("Molly Mosquito") is the most commonly detected substituted amphetamine and has similar entactogen effects as MDMA (Molly). An association between the use of substituted amphetamines and acute cardiomyopathy has been previously described. The most frequently reported clinical effects of modafinil and methylphenidate overdoses are generally mild, predominately tachycardia. However, there is little data on fluoroamphetamines, methylphenidate, or modafinil causing acute cardiomyopathy, other than single case reports.

Case: An 18 year old female presented to the emergency department (ED) after reportedly ingesting 800mg of modafinil, insufflating 100mg of methylphenidate, and drinking two capfuls of "Molly's Mosquito cap." She reported initial euphoric effects, and then headache, nausea, vomiting, lightheadedness, and diaphoresis. Her only complaint was anxiety upon arrival at the ED. An echocardiogram obtained approximately 36 hours after ingestion showed a cardiac ejection fraction of 10-15%, with mild left ventricular dilation and severe diffuse hypokinesis. Forty-eight hours after admission she had mild tachycardia, though her symptoms had improved and she was discharged. The normal adult therapeutic doses for modafinil and methylphenidate are 200-400mg/day and between 20-50mg/day, respectively.

Objective: The objective of this study was to develop a method for the detection and quantification of modafinil, methylphenidate, and its primary metabolite ritalinic acid in urine in clinical cases where their abuse is suspected.

Method: An ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method was developed for the detection and quantitation of modafinil, methylphenidate, and its primary metabolite ritalinic acid in urine. A six-point calibration curve ranged from 25-1000ng/mL and five quality control specimens (50, 150, 400, 800, and 2500 ng/ml) were analyzed on each day. Following the addition of the deuterated internal standards (modafinil-d10, methylphenidate-d9, ritalinic acid-d4), specimens were extracted by solid phase extraction using a mixed mode column, following a method for the extraction of acidic and basic compounds (UCT Clean Screen® DAU). Analysis was performed using a Waters Acquity Xevo TQD LC-MS/MS with EI ionization in positive mode.

Result: The urine concentration of modafinil was 1.08mg/L (1080ng/mL), and methylphenidate and ritalinic acid were both <0.10mg/L (<100ng/mL). The 4-fluoramphetamine urine concentration was 37,000ng/ml, determined previously. The linear regression correlation coefficients for each analyte's calibration r^2 were 0.992 or greater. The limit of quantitation (LOQ) was administratively set at 50ng/mL. Accuracy/bias of the assay was determined to be within +/-20% of the target value for each analyte in each quality control specimen. The CV for inter-day and intra-day precision samples did not exceed 15%, except modafinil at the LOQ. Validation criteria for matrix effects, absolute recovery, carryover and specificity were acceptable.

Conclusion/Discussion: Acute dilated cardiomyopathy occurred in a young female exposed to several substances, including modafinil, methylphenidate, and 4-fluoramphetamine. The high doses of modafinil and methylphenidate taken by the patient may have contributed to the acute dilated cardiomyopathy that has been associated with substituted amphetamines. The presented method is sensitive and robust for the determination of modafinil, methylphenidate and its primary metabolite ritalinic acid in urine.

Keywords: Modafinil, Methylphenidate, Ritalinic Acid

S13

Toxicological Confirmation of Intoxications Involving the Designer Benzodiazepine Clonazolam

Melissa Friscia*[1], Heath A. Jolliff[2], Barry K. Logan[3]; [1]Center for Forensic Science Research and Education, Willow Grove, PA, [2]Mid-Ohio Toxicology Services, LLC, Columbus, OH, [3]NMS Labs Willow Grove, PA

Background/Introduction: The recreational drug market has seen a proliferation of synthetic drugs, many of which are analogs or minor chemical modifications of existing compounds. This has led to a large number of synthetic cannabinoids, cathinones and beta-keto amphetamines, and most recently opioid receptor agonists entering the illicit drug market. The latest pharmacological class to be impacted in this way are the benzodiazepines. Various novel benzodiazepines, which have never been available legitimately in the United States, are now appearing in the illicit drug supply. Among the first benzodiazepines identified in controlled substance casework were phenazepam and etizolam, followed more recently by flubromazepam, flubromazelam, bromazepam, and clonazolam. Clonazolam is a potent triazolo-analog of clonazepam, and is also known as clonitrazolam, with a 0.5 mg oral dose leading to strong sedation and amnesia.

Objective: The purpose of this presentation is to describe the identification of clonazolam in "Pinzor" tablets, and report a method for screening and confirmation of clonazolam in urine from two individuals who appeared in the emergency room following the alleged ingestion of the tablets.

Method: Screening analysis was performed using liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF) (Xevo® G2-S with an Acquity I-class UPLC, Waters®; Milford, MA) to establish the presence of drugs and their accurate mass. The method has been previously described and validated against an in house database for approximately 1200 drugs and their common metabolites, including medicinal, therapeutic and novel psychoactive substances. Confirmatory analysis for clonazolam was performed by liquid chromatography tandem mass spectrometry (LC-MS/MS) (Agilent 6430 with a 1200 Series HPLC, Santa Clara, CA). Confirmatory methods for urine were validated according to SWGTOX guidelines, including determination of linear range, limits of detection and quantitation, precision, accuracy, stability, and interferences. Additional confirmatory analysis for clonazolam in the tablets was performed by gas chromatography mass spectrometry (GC/MS) (Agilent 5975; Santa Clara, CA).

Result: The analysis of the "Pinzor" tablets revealed the presence of a single major peak with an accurate mass of 354.0779, consistent with $C_{17}H_{12}ClN_5O_2$, the molecular formula for clonazolam. This identification was confirmed by verification of retention time, and GCMS analysis relative to a clonazolam reference standard. The methods were used to screen urine samples from two subjects reporting to an emergency room with symptoms of acute intoxication. Subject 1 was a 20-year-old male, who has a reported history of anxiety and depression. He was found unconscious in his car. He admitted to taking three 0.7 mg tablets of a "Pinzor" that had been purchased on-line. Upon arrival to the hospital he had stable vital signs, but quickly became bradycardic and hypotensive. The urine drug screen (UDS) performed at the hospital had produced a positive result for benzodiazepines. We detected and confirmed the presence of clonazolam in the urine at a concentration of 15.3 ng/mL.
Subject 2 was an 18-year-old male from the same vehicle who admitted to taking two 0.7 mg "Pinzor" tablets. He described becoming drowsy after taking the pills and was awakened by emergency medical services. His vitals were significant for tachycardia and hypertension. The UDS performed in the hospital produced positive results for benzodiazepines, THC and opiates. We detected and confirmed the presence of clonazolam in the urine at a concentration of 30.7 ng/mL.

Conclusion/Discussion: This is the first known clinical report of a clonazolam intoxication in the emergency room in the United States and confirms concerns that novel benzodiazepines may now be available in the United States. The potency of this drug creates concerns about forensic implications for its use and should be considered as a possibility in cases with positive immunoassay benzodiazepine results that do not confirm for more common benzodiazepines.

Keywords: Designer Benzodiazepines, Clonazolam, LC-MS/MS

S14
An Overdose Death Involving Designer Benzodiazepine Flubromazolam

Kevin J. Schneider, Tiffany M. Leusby, **Bonnie Y. Lo***, Juli A. Cruciotti, Carol L. O'Neal; Virginia Department of Forensic Science, Northern Laboratory, Manassas, VA

Background/Introduction: Flubromazolam is an extremely potent designer benzodiazepine that bears structural resemblance to flubromazepam and triazolam. While many warnings exist as to the high potency and danger associated with flubromazolam, very few case studies appear in the published literature regarding this drug. Here we describe a lethal overdose case involving both flubromazolam and heroin. Case history states that an individual ingested small round candies potentially adulterated with drugs during the evening and became unresponsive. Throughout the night the individual required resuscitation from friends multiple times. The following morning, the patient was admitted to the hospital in an unresponsive state, surviving numerous hours with the assistance of life support. Eventually, the patient expired and a death investigation was initiated. Specimens were submitted to the Department of Forensic Science Toxicology section for testing and the remaining candies were submitted to the Controlled Substances section.

Objective: The objective of this case study was to investigate the toxicological components that played a role in the events leading to the death of the decedent. In particular, there was interest in the possible presence of the potent benzodiazepine flubromazolam and the concentration in biological specimens resulting from ingestion.

Method: The Controlled Substances section qualitatively determined the remaining candies to contain flubromazolam and heroin, prompting the Toxicology section to develop a method for the analysis of flubromazolam. Mass spectral parameters for flubromazolam were optimized using the Agilent Optimizer software program and a purchased flubromazolam reference material. Benzodiazepine quantification was performed using an alkaline extraction with 0.2 M sodium carbonate and 1-chlorobutane. The organic phase was evaporated to dryness under nitrogen and reconstituted with methanol. Extracts were analyzed via LC-MS/MS using dynamic multiple reaction monitoring (MRM) mode. The calibration range for flubromazolam was 0.010 – 0.50 mg/L. Numerical values obtained using this method were semiquantitative because the method was not fully validated for flubromazolam according to SWGTOX guidelines.

Result: Specimens submitted for toxicological testing included hospital serum and urine from the time of admission and whole blood collected ~7h later. Approximate flubromazolam concentrations in serum, urine, and whole blood were 0.018, 0.048, and 0.020 mg/L, respectively. Additional findings in the serum included morphine at 0.019 mg/L and the presence of morphine, codeine, and 6-acetylmorphine was confirmed in the urine.

Conclusion/Discussion: This case documents the first instance of an overdose death associated with flubromazolam in the literature. While other documented cases did not involve the toxicological interplay of flubromazolam with opiates as in this case, the symptomology among cases is similar: severe, long-term depression of the central nervous system up to/including deep coma, respiratory depression with possible cardio-respiratory failure, and possible hypoxic-ischemic changes to the brain. The potency of flubromazolam is evident from existing literature, showing that dosage at 0.043 mg/kg (3 mg/70 kg) is sufficient to produce deep coma and cardio-respiratory failure on its own.[1] The results of this investigation provide key associations between toxicological analyses and case history that will assist toxicologists as the designer benzodiazepine flubromazolam emerges into the recreational market.

Keywords: Flubromazolam, Overdose, Benzodiazepine

S15
Profiling Metabolites of Cocaine Adulterants in Urine

Sarrah Hannon*[1], Adam B. Hall[2]; [1]Harris County Institute of Forensic Sciences, Houston, TX, [2]Barnett Institute of Chemical and Biological Analysis- Northeastern University, Boston, MA

Background/Introduction: In recent years data from the National Forensic Laboratory Information System (NFLIS) has demonstrated the steady decrease in purity of certain illicit drugs, such as heroin and cocaine. National agencies have been studying the other contents of illicit drug samples for many years through programs such as the Cocaine Signature Program (CSP) through the Drug Enforcement Administration (DEA) (1). There are many studies for the diluents and adulterants commonly present in seized drug samples, but if the drug of interest is not available the biological samples can indicate other ingredients in the illicit drug. The following study analyzed common cocaine adulterants and/or their metabolites in benzoylecgonine positive urine samples in a large pain management lab (Calloway Laboratories) in Massachusetts.

Objective: By studying the metabolites in urine or other biological samples, information about the abused drug can be ascertained, even when the drug was not or could not be seized as part of the investigation. Often times toxicology labs, specifically performing work for medicolegal death investigation purposes, report only the drugs present with significant impact on the impairment or eventual fatality of the victim. Common adulterants of cocaine include, but are not limited to the following: lidocaine, procaine, benzocaine, caffeine, and levamisole. These adulterants and their metabolites are detected but typically not reported. But, each of these metabolites carries their own risks and possible fatal complications. By performing this sort of adulterant analysis, more information could be provided for pathologists and profiling of adulterants could be more accessible. The objective of this research was to study lidocaine, levamisole and di-ethylaminoethanol (metabolite of procaine) in benzoylecgonine positive urine samples by solid-phase extraction (SPE) and subsequent analysis by Liquid Chromatography/tandem Mass Spectrometry (LC-MS/MS) to determine state and regional profiles of common cocaine adulterants.

Method: 350 urine samples were extracted and analyzed to identify and quantitate three different cocaine adulterants: procaine (identified by metabolite-di-ethylaminoethanol), lidocaine and levamisole. The patient samples were acquired after completion of analysis at Calloway Labs, a pain management laboratory. The state of origin and the quantitative value of benzoylecgonine were collected at the time of sample selection. 28 states were represented in five different regions allowing for a regional profile of these three adulterants.

One milliliter (mL) of sample was extracted using Clean Screen extraction columns from United Chemical Technologies (UCT); no internal standard was used. The samples were then analyzed utilizing an Agilent 50 millimeter (mm) C18 Zorbax Eclipse Plus with an internal diameter of 4.6 mm and a pore size of 1.8 micrometers. Mobile phase A consisted of 2% acetonitrile with 0.1% formic acid and 2 millimolar ammonium acetate and mobile phase B consisted of 0.1% formic acid in acetonitrile. The MS/MS instrument method utilized multiple reaction monitoring (MRM) mode with the following transitions:

Compound	Molecular Ion	Fragment Ion
Di-ethylaminoethanol	118.1	72.1
Levamisole	205.0	178.1
Lidocaine	235.2	86.0

Result: Of the 350 samples, 340 (97%) were positive for levamisole, 85 (24%) were positive for di-ethylaminoethanol and 63 (18%) were positive for lidocaine. Five regions were defined for the purposes of profiling: Mid-Atlantic, Midwest, Northeast, Southeast and Southwest. The Midwest showed a higher percentage of di-ethylaminoethanol positive samples while the southern regions showed a higher number of positive results for lidocaine. When compared state by state, even levamisole showed a small trend with slightly smaller percentages of positive samples coming from Mid-Atlantic, Midwest and Northeast regions. The method was quantitative with varying linear ranges for each compound.

Conclusion: Levamisole as an adulterant still appears to be on the rise. The percentage of levamisole positive cocaine (97%) has risen from 77% reported in 2010 from a study of benzoylecgonine positive urine samples in Denver, Colorado (2). A state by state analysis, within regions, showed specific trends within the regions and across all five regions studied for the other two metabolites, lidocaine and procaine. These trends could be because of availability of the adulterants or actual regional trends. The results of this study provide more evidence toward the growing epidemic of levamisole as an adulterant but also show that profiling of cocaine adulterants in biological samples can be performed and provide trends and results of interest.

(1) U.S. Drug Enforcement Administration. National Forensic Laboratory Information System; 2011 Annual Report, 2012.
(2) Buchanan, J, et. al. Research Letter: Prevalence of Levamisole in Urine Toxicology Screens Positive for Cocaine in an Inner-City Hospital. *Journal of the American Medical Association*2011; 305 (16): 1657-1658.

Keywords: Levamisole, Cocaine, Adulterants

S16

Profiles of Synthetic Cannabinoids Metabolites in Body Tissues - A Fatal Case Report

Bogdan Tokarczyk*, Dominik Bakalarz, Bogumila Byrska; Institute of Forensic Research, Krakow, Poland

Background/Introduction: In recent years, a rise in the use of new synthetic cannabinoids (SC) has been observed. It is the fastest developing group of new psychoactive substances. Unfortunately, their use has resulted in many serious poisonings and fatal cases such as the one presented here. A 20-year-old male was in a pub drinking alcohol with friends and they went outside to smoke. Later that night he was found dead sitting on a toilet seat. A plastic bag containing 4.76 g of green herbal blend and a cigarette pipe were found next to him. The autopsy was conducted about 2 days later. Blood samples, lung, brain and liver tissue, as well as the pipe and herbal blend were delivered for toxicological analysis.

Objective: The aim of the study was to detect metabolites of AB-PINACA, 5F-AB-PINACA and 5F-AMB in body tissues and to investigate their distribution in the human body.

Method: Herbal material was homogenized and 0.2 g of the sample was subjected to extraction with methanol. It was analyzed by GC-MS, UPLC-DAD and LC-MS/MS. The blood (0.5 mL) and body tissues (5 g) were analyzed for the presence of main metabolites of synthetic cannabinoids. Amphetamine-D5 was added to the samples as an internal standard. The analytes were isolated from blood by acetonitrile precipitation. A homogenization with methanol/water following by an extraction with dichloromethane/methanol was performed to isolate metabolites from body tissues. Extracts were evaporated and reconstituted in mobile phase (A:B–1:1). Analyses were carried out using a 3200 QTRAP LC-MS/MS. Separation was performed on a Phenomenex Kinetex C18 (3.0x50 mm, 2.6 μm particle size) column using gradient elution of acetonitrile/methanol (B) and 1% (v/v) ammonium formate in water (A). A whole blood linear calibration curves for parent molecules ranged from 0.5 to 50 ng/mL. The LOD and LOQ were determined to be 0.2 ng/mL and 0.5 ng/mL, respectively. Multiple reaction monitoring (MRM) with positive ion detection was used. Two MRM transitions each for AB-PINACA (331→215/286), 5F-AB-PINACA (349→233/304), 5F-AMB (364→233/304) and 5F-AKB-48 (384→93/135) were monitored.

Result: The analyses of herbal material revealed AB-PINACA, 5F-AB-PINACA, 5F-AMB and 5F-AKB-48 in the concentrations 12, 9, 27 and 0.4 mg/g, respectively. Their presence was also confirmed in the cigarette pipe. AB-PINACA and 5F-AB-PINACA were determined in the blood in the concentrations 15.0 and 6.4 ng/mL, respectively, as well as the traces of 5F-AMB were found (concentration lower than 0.5 ng/mL). 5F-AKB-48 was not detected. AB-PINACA was found in all analyzed tissues. Four metabolites of AB-PINACA were identified in blood. These substances are products of: oxidation (AB1), amide hydrolysis (AB2), amide hydrolysis followed by ketone formation (AB3) and amide hydrolysis followed by oxidation (AB4). The same compounds, excluding AB3, were identified in the lung and liver. Only AB4 was detected in brain tissue. Unfortunately, products of 5F-AB-PINACA hydrolysis followed by oxidation, defluorination and carboxylation are also 5F-AMB metabolites, so they cannot be distinguished by means of applied method. In this case, three common metabolites were found, which derive from: amide and ester hydrolysis (5F1), oxidative defluorination (5F2) and oxidative defluorination followed by carboxylation (5F3). Product of 5F-AB-PINACA oxidative defluorination (5FM1) was also present. No traces of adequate 5F-AMB metabolite was found. Comparing relative intensities it can be concluded that forming of AB4 and AB3 is the most efficient metabolic pathway of AB-PINACA. In case of 5F-AB-PINACA and 5F-AMB, similar metabolic pattern can be observed resulting primarily in formation of 5F1 and 5F2.

Conclusion/Discussion: Main metabolites AB-PINACA, 5F-AB-PINACA and 5F-AMB were detected and identified in body tissues. 5F-AB-PINACA (found only in blood) and 5F-AMB were not detected, which was surprising, concerning their high concentration in the seized herbal blend. Lack of the mentioned compounds can suggest fast metabolism. Concerning very low active doses, even less than 1 mg, resulting in low blood concentration, identified metabolites seems to be good candidates for markers to confirm presence of parent molecules. Evaluation of SC metabolites profile appeared to be useful tool in metabolite path examination.

Keywords: Synthetic Cannabinoids, Postmortem Toxicology, LC-MS QTRAP

S17

Methamphetamine, Amphetamine, and Norephedrine Levels in Dermestid Beetle Frass after Consumption of Dosed, Buried Rat Remains

Meaghan P. Drumm*[1], Kimberlee S. Moran[1], M. Lee Goff[2], Karen S. Scott[1]; [1]Arcadia University, Glenside, PA, [2]University of Hawaii at Manoa, Honolulu, HI

Background/Introduction: Methamphetamine is a Schedule II drug that is associated with a high rate of death in users who abuse this substance. Traditionally, blood and urine are used to determine the presence of drugs in postmortem casework; however, in cases where a body is badly decomposed, alternative matrices may be required. Insects are known to consume human remains when a death has occurred outdoors. There are multiple studies detecting various drugs within blowflies (order Diptera). Few studies have attempted to detect and quantify drugs within other insects such as Dermestid beetles (order Coleoptera). These beetles are the last insects found feeding on decomposing remains. To date, the only reported studies dealing with Coleoptera have been on beetle exuviae, from which amitriptyline and nortriptyline were isolated.

Objective: This research evaluates Dermestid beetle frass for detecting methamphetamine and its metabolites and assesses the viability of this matrix for toxicological analysis. This research also identifies if dose and varying levels of decomposition, determined by calculating accumulated degree days (ADDs), has any effect on the final concentration of the drugs within the beetle matrix.

Method: Rats dosed with 5 mg/kg (n=4), 3 mg/kg (n=4), 2.5 mg/kg (n=2), 1.5 mg/kg (n=2), 0.5 mg/kg (n=4), and 0.25 mg/kg (n=2) methamphetamine were euthanized and buried. Control rats were injected with saline and buried (n=3) or un-injected and not buried (n=2). Buried specimens were exhumed at different decomposition stages (89, 182, 395, and 819 ADDs) to determine effects of decomposition on the ability to detect the drugs using GC-MS. After exhumation or euthanization without burial (un-injected and not buried controls), rats were dissected, pelted, and dried before being fed to Dermestid beetles (*Dermestidae maculatus*). Eight adult beetles were placed on each sample until they laid eggs. The adults were then removed. The offspring consumed meat of the dosed rats until full skeletonization had occurred. Beetle frass was collected, incubated in phosphate buffer containing β-glucuronidase for 2 hours at 40°C, then centrifuged and extracted using solid phase extraction. The amounts of methamphetamine, amphetamine, and norephedrine were quantified in all beetle media using GC-MS with methamphetamine-D5 and amphetamine-D5 as internal standards.

Result and Conclusion/Discussion: We determined that methamphetamine could be detected in the frass of Dermestid beetles fed on buried, dosed rat remains. For methamphetamine, concentrations were found to range from <0.001 to 1.07 ng/mg frass; for amphetamine, concentrations ranged from <0.001 to 0.465 ng/mg; and for norephedrine, concentrations ranged from <0.001 to 0.025 ng/mg. All un-drugged controls were found to be negative. No correlation was observed between rats receiving the same dose at different ADDs or rats receiving increasing doses at the same exhumation time.

Keywords: Methamphetamine, GC-MS, Entomotoxicology

S18

Cannabinoid Pharmacokinetics and Detection Windows in Oral Fluid after Controlled Smoked, Vaporized, and Oral Cannabis Administration

Madeleine J. Swortwood*[1], Matthew N. Newmeyer[1,2], Osama A. Abulseoud[1], Karl B. Scheidweiler[1], Marilyn A. Huestis[1,3]; [1]Chemistry and Drug Metabolism, IRP, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD, [2]Program in Toxicology, School of Medicine, University of Maryland Baltimore, Baltimore, MD, [3]University of Maryland School of Medicine, Baltimore, MD

Introduction: Oral fluid (OF) is an important matrix for monitoring driving under the influence of drugs (DUID). Although smoking is the most common cannabis administration route, vaporization and oral consumption via edibles are becoming popular. OF pharmacokinetics after smoked and vaporized cannabis and oral Δ^9-tetrahydrocannabinol (THC) administration are well-characterized. However, there are few OF pharmacokinetic data after oral edible cannabis, and direct comparison of cannabinoid pharmacokinetics after these routes has not been investigated. Furthermore, OF detection windows for parent cannabinoids and metabolites need to be established, particularly after oral cannabis administration.

Objective: To characterize OF cannabinoid disposition and detection windows following smoked, vaporized, and oral cannabis administration in frequent and occasional cannabis smokers.

Method: Eleven frequent (≥5x/week) and nine occasional (≥2x but <3x/week) cannabis smokers were recruited to participate in this National Institute on Drug Abuse Institutional Review Board-, FDA-, and DEA-approved study; all participants provided written informed consent. The study was randomized, double-blind, and placebo-controlled with a crossover and double-dummy design. Over 4 dosing sessions, participants were administered one active (6.9% THC; 54mg) or placebo cannabis-containing brownie followed by one active or placebo cigarette or one active or placebo vaporized cannabis dose. No more than one active dose was administered per session. OF was collected with Quantisal™ collection devices before and up to 54 or 72h after dosing from occasional and frequent smokers, respectively. THC, 11-hydroxy-THC (11-OH-THC), 11-nor-9-carboxy-THC (THCCOOH), tetrahydrocannabivarin (THCV), cannabidiol (CBD), and cannabigerol (CBG) were quantified by a validated liquid chromatography-tandem mass spectrometry method with 0.2µg/L limits of quantification (except 15ng/L THCCOOH). Maximum concentration (C_{max}), time to C_{max} (t_{max}), and time of last detection (t_{last}) were determined. Repeated-measures ANOVA was calculated for overall dose effect with planned Helmert contrasts.

Result: Significantly greater median (range) THC C_{max} were observed after smoked (1985[141-8503]µg/L) and vaporized (437[68.6-7373]µg/L) cannabis versus oral (133[16.4-938]µg/L) administration in frequent smokers. Significantly later THCV, CBD, and CBG t_{last} were observed after smoked (4.7, 8.1, 10.6h, respectively) and vaporized (3.9, 7.4, 5.4h, respectively) cannabis compared to oral (1.7, 2.2, 3h, respectively) cannabis in frequent smokers. No significant differences in THC, 11-OH-THC, THCV, CBD, or CBG t_{max} between routes in frequent or occasional smokers were observed. 11-OH-THC and THCCOOH were detected in more occasional smokers after oral (66.7% and 100%, respectively) cannabis administration than after smoked (33.3% and 55.6%, respectively) and vaporized (0% and 33.3%, respectively) routes, increasing % positive >LOQ and widening metabolite detection windows after oral dose consumption.

Conclusion: Cannabinoids and metabolites were fully characterized in OF following controlled cannabis brownie consumption with direct comparison to smoked and vaporized administration. OF cannabinoid C_{max} occurred immediately after cannabis consumption, regardless of route, due to oral mucosa contamination, with no significant differences in t_{max}. However, significantly greater OF THC concentrations were observed following inhaled cannabis than after oral administration. THCV, CBD, and CBG were detected significantly longer after inhaled cannabis in frequent smokers. Detection of 11-OH-THC in OF indicated recent cannabis intake (within 1.5h). Utilizing THCV, CBD, and CBG cutoffs (0.3-0.5µg/L) resulted in detection windows indicative of recent cannabis intake (within 20h, depending on administration route), regardless of smoking history.

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

Keywords: Oral Fluid, Cannabinoids, Edibles

S19

Optimisation of a GC-MS Method for the Quantitative Identification of 23 New Psychoactive Substances in Blood and Urine

Lorna A. Nisbet*[1, 2], Barry K. Logan[3], and Karen S. Scott[2]; [1]Forensic Medicine and Science, University of Glasgow, Scotland, UK; [2]Forensic Science, Arcadia University, Glenside, PA; [3]Center for Forensic Science Research and Education, Willow Grove, PA

Background/Introduction: New psychoactive substances (NPSs) have become an integral part of the recreational drug market with "new" compounds being reported by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) biweekly. NPSs pose an unprecedented threat due to the speed at which they appear on the recreational drug market. By the time a substance is detected, reference standards become available, and legislation is passed, a new compound has already taken its place.

Objective: The objective of this study was to develop and validate a quantitative GC-MS method for the detection of 2-DPMP, 3-MeO-PCE, 3-MeO-PCP, 5-APB, 6-APB, benzedrone, butylone, ethylone, flephedrone, methiopropamine, MDPV, mephedrone, methoxetamine, methylone, naphyrone, 25B-NBOME, 25C-NBOME, 25D-NBOMe, 25E-NBOME, 25H-NBOME, 25I-NBOME, mescaline-NBOME, and 25P-NBOME—predominantly cathinones and NBOMes, allowing for their simultaneous detection and quantification from blood and urine samples.

Method: Sample preparation was carried out using UCT CLEANSCREEN CSDAU SPE cartridges. Column conditioning was done by the addition of 3 mL MeOH, 3 mL dH_2O, and 1 mL pH 6 phosphate buffer. To samples, 3 mL of pH 6 phosphate buffer was added, along with 0.5 mg/L internal standard (mephedrone-D3, methylone-D3, ethylone-D5, and MDPV-D8) and 0.5 µg/L 25I-NBOMe-D3. Samples were then centrifuged for 10 minutes at 4500 rpm. The supernatant from each sample was transferred to SPE columns and allowed to pass through. Column washing was carried out with 3 mL dH_2O, 1 mL acetic acid (1 M), and 3 mL MeOH, after which the cartridges were left to dry fully under full vacuum. Elution was carried out using 3 mL of DCM/IPA/NH_3 (78/20/2), followed by evaporation under a gentle stream of nitrogen and derivatization using PFPA:EtOAc (2:1) for 20 minutes at 37ºC. Samples were then evaporated to dryness, reconstituted using 50 µL of EtOAc, and analyzed by GC-MS. The GC-MS was operated in SIM mode with an EI source and a splitless injection. The injection port temperature was 225ºC, and the transfer line temperature was 250ºC with an MS source temperature of 200ºC. The initial oven temperature was 80ºC, which was held for 2 minutes, before ramping to 170ºC at a rate of 25ºC/minute and being held for 1 minute. The temperature was then further increased to 200ºC at 5ºC/minute and held for 1 minute before being ramped at 15ºC/minute to 250ºC. Finally, the oven temperature was increased to 300ºC at a rate of 5ºC/minute and held for 3 minutes. The total run time was 30 minutes. Calibrators and controls were analysed alongside samples.

Result: Parameters investigated for validation included bias, linear calibration model, carryover, interferences, limit of detection, limit of quantification, precision, and stability. All drugs provided successful results for each of these parameters as per SWGTOX guidelines. The GC-MS method was used for the reanalysis of 12 blood samples (8 cases) where 25I-NBOMe, 25C-NBOMe, methoxetamine, and methylone had previously been detected by NMS Labs. Of these samples, this GC-MS method was able to quantitatively detect these drugs in 9/12 blood samples, 5 of which contained either 25C-NBOMe or 25I-NBOMe. Concentrations detected fell within ±20% for all samples with the exception of case 2 sample A, which saw a 22.1% decrease in 25C-NBOMe detected (1.69 µg/L vs. 2.17 µg/L) and case 8 sample A, which saw a 35.3% decrease in methylone detected (0.11 mg/L vs. 0.17 mg/L). No other substances were detected in these samples.

Conclusion/Discussion: A robust and sensitive GC-MS method was developed for the detection of various NPSs in blood and urine. This method is of particular use for laboratories who do not possess an LC-MS/MS for the detection of NBOMes in acute fatalities. Further testing for this method should be carried out as more case samples become available.

Keywords: NPSs, NBOMes, GC-MS

S20
Metabolomics in Urine: Seroquel® and Beyond

Erin C. Strickland*, Oneka T. Cummings, Gregory L. McIntire; Ameritox, Ltd., Greensboro, NC

Background: Monitoring of adherence to antipsychotic medications is key to adequate patient care. Urine drug monitoring is employed to better inform the clinician about patient adherence with his/her therapy. Therefore, the drug and metabolites selected to monitor adherence are crucial so as to not improperly induce a clinician to make an unwarranted change to a patient's therapy. Quetiapine (Seroquel®) data showed poor correlation between prescriptions and positivity rate (apparent adherence) and were investigated to determine if there was a more prevalent metabolite(s) that could be used for monitoring prescription use.

Objective: Determine if there were additional or alternative metabolites that could be used to monitor Seroquel® use with improved apparent adherence rates from the existing method.

Method: Samples acquired from patients who were prescribed quetiapine and tested positive on the existing method were selected to be run on an Agilent 6530 Q-TOF. Samples were diluted 5x with hydrocodone-d6 in water and run on a generic 4 minute gradient using a Phenomenex Phenyl-Hexyl 2.6 µm 2.1 x 50 mm column. Samples were run in MS-only and MS/MS mode. Samples were then searched for the presence of all known and suspected metabolites using Mass Hunter Qualitative Analysis software. Potential metabolite hits were curated based on overall score (mass accuracy, isotopic distribution, and isotopic spacing based on theoretical values from the chemical formula), retention time consistency across samples, and abundance. After identifying the strongest candidate metabolites, standards were acquired, if possible, to confirm identity and develop a LC-MS/MS MRM method for validation and patient monitoring.

Result: Quetiapine and 7-hydroxyquetiapine are validated and currently used to monitor Seroquel® usage in patients and yield an apparent adherence rate of approximately 31%. Data from the Q-TOF analysis indicated that quetiapine carboxylic acid was more prevalent in patient samples and could be a better metabolite marker for Seroquel® use. Additionally, quetiapine sulfoxide and norquetiapine were observed at about half the abundance of the carboxylic acid but were not consistently identified in every patient. Using the quetiapine carboxylic acid alone to measure the apparent Seroquel® adherence, the rate increased to approximately 47%. Although quetiapine glucuronide and 7-hydroxyquetiapine glucuronide were not initially identified as prevalent metabolites based on the results from the Q-TOF analysis, hydrolysis provided a significant increase in the abundance of both quetiapine and 7-hydroxyquetiapine in urine. It was noted that on average the hydrolysis increased the abundance of quetiapine by 95% and the abundance of 7-hydroxyquetiapine by 96%. This elevation in prevalence of quetiapine and 7-hydroxyquetiapine after hydrolysis led to an increase in the apparent adherence rate to 41% when using these analytes alone to determine apparent adherence. Overall, with the use of hydrolysis and the addition of the quetiapine carboxylic acid the apparent adherence reached a high of 50%.

Conclusion/Discussion: Various published studies have placed the adherence rate for antipsychotic medications, including Seroquel® anywhere from 30-52%, depending on the disease state of patients. With the improvements that hydrolysis and the addition of the quetiapine carboxylic acid metabolite provide, the apparent adherence rate of the method correlates in the higher percentage range of the published studies. Not only did this provide a benefit to this specific application, but it created a paradigm that can be used and expanded to other antipsychotics, drugs, or matrices to ensure the highest fidelity of results.

Keywords: Metabolomics, Hydrolysis, Quetiapine

S21
Drug Recognition Expert (DRE) Examination Characteristics of Cannabis Impairment

Rebecca L. Hartman*[1], Jack E. Richman[2], Charles E. Hayes[3], Marilyn A. Huestis[1,4]; [1]Chemistry and Drug Metabolism, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD, [2]Hingham Police Department, Hingham, MA, [3]International Association of Chiefs of Police, Alexandria, VA, [4]Huestis & Smith Toxicology, LLC, Severna Park, MD

Background/Introduction: The Drug Evaluation and Classification Program (DECP) is commonly utilized in driving under the influence (DUI) cases to help determine category(ies) of impairing drug(s) present in drivers. Cannabis, one of the categories, is associated with approximately doubled crash risk.

Objective: To determine the most reliable DECP metrics for identifying cannabis-driving impairment.

Method: We evaluated 302 toxicologically-confirmed (blood Δ^9-tetrahydrocannabinol [THC] ≥1 µg/L) cannabis-only DECP cases, wherein examiners successfully identified cannabis, compared to normative data (302 non-impaired individuals). Drug Recognition Experts (DREs) utilized the standardized 12-step DECP evaluation system to assess drivers (cases) and non-impaired volunteers (controls). Physiological measures, pupil size/light reaction, and performance on psychophysical tests (e.g., one leg stand [OLS], walk and turn [WAT], finger to nose [FTN], Modified Romberg Balance [MRB]) were included.

Result: Cases were significantly younger than controls (median [range] ages 21 [15-59] and 34 [15-59], respectively, with no difference in sex distribution (cases, 87.4% M/12.6% F; controls, 89.2% M/10.8% F). Cases significantly differed from controls ($p<0.05$) in pulse (increased; 91 [49-166] bpm versus 71 [39-107 bpm) and systolic blood pressure (elevated; 138 [82-205] versus 130 [90-170]). Blood collection time after arrest was associated with significantly decreased THC concentrations; no significant differences were detected in performance measures between cases with blood THC <5 µg/L and ≥5 µg/L. Cases' mean (SD) pupil size was significantly more dilated than controls ($p<0.001$). Horizontal gaze nystagmus (HGN) occurrence did not significantly differ between cases and controls (2.65% versus 0.33%), but lack of convergence (LOC) and rebound dilation occurred significantly more ($p<0.001$) in cases (78.8% and 70.9%, respectively) than controls (10.9% and 1.0%). For the OLS, 55.0% of cases demonstrated ≥2 of 4 possible impairment clues on at least one leg. The median number of observed clues on either leg was significantly greater for cases (1) than controls (0). Of 8 possible impairment clues on the WAT, 3 [0-8] were detected for cases and 0 [0-2] for controls ($p<0.001$). Cases missed substantially more than controls (5 [0-6], 0 [0-6] of 6 possible misses respectively, $p<0.0001$) on the FTN. MRB observations included broader, non-normal distribution of cases' estimation of 30 s than controls' (29 [4-90] versus 30 [20-53], $p=0.002$). The FTN best predicted cannabis impairment (sensitivity, specificity, positive/negative predictive value, and efficiency ≥87.1%) when utilizing ≥3 misses as the deciding criterion; MRB eyelid tremors produced ≥86.1% for all diagnostic characteristics. Other strong indicators included OLS sway, ≥2 WAT clues, and pupil rebound dilation. Requiring ≥2/4 of: ≥3 FTN misses, MRB eyelid tremors, ≥2 OLS clues, and/or ≥2 WAT clues produced the best results (all characteristics ≥96.7%).

Conclusion/Discussion: Blood specimens should be collected as early as possible. The frequently-debated 5 µg/L blood THC per se cutoff showed limited relevance to test performance. Combined observations on psychophysical and eye exams (including elevated pulse, dilated pupils, LOC, rebound dilation, and documented impairment in ≥2 of 4 psychophysical tasks) produced the best cannabis-impairment indicators.

Research funded [in part] by the Intramural Research Program, NIDA, NIH.

Keywords: Drug Recognition Expert, Drug Evaluation and Classification Program, Cannabis

S22
Characteristics of Alcohol-Impaired Drivers in the City of Houston

Dayong Lee*, Peter Stout; Houston Forensic Science Center, Inc., Houston, TX

Background/Introduction: Ethanol (Alcohol) is one of the oldest and the most prevalent drug of abuse in the United States and worldwide. An estimated 139.7 million Americans aged 12 or older (52% of the population) reported alcohol use in the past month in 2014. Alcohol-impaired driving fatalities, defined as fatal crashes involving drivers with a blood alcohol concentration (BAC) ≥ 0.08 g/dL, accounted for 31% of all fatal motor vehicle crashes in the U.S. in 2014; Texas had the highest alcohol-impaired driving fatalities (n=1,446) and also the highest percentage of such fatalities in total fatal crashes (41%) among all 50 states. Houston is the most populous city in Texas and the fourth most populous city in the U.S.

Objective: This study examines BAC and demographic characteristics of individuals involved in alcohol-impaired driving in the city of Houston. The distribution of BACs and risk groups associated with alcohol-impaired driving are evaluated to aid in designing and implementing regulations and prevention efforts to reduce this serious public safety issue.

Method: The driving while intoxicated (DWI) or driving under influence of drug (DUID) cases, fatal and non-fatal, analyzed by Houston Forensic Science Center for alcohols in blood samples from January 1, 2015 to February 19, 2016 were included. The samples were submitted by the Houston Police Department and collected from drivers or suspects as indicated on the laboratory information management system. The data were further filtered to include only the cases with the offense occurring in 2015 or 2016. BAC and demographics including age, sex, and race/ethnicity were evaluated. Ethanol along with methanol, acetone, and isopropanol was analyzed utilizing headspace gas chromatography interfaced with a flame ionization detector. The limit of quantification (LOQ) was 0.010 g/dL and range of linearity was 0.010-0.500 g/dL for ethanol (0.010-0.400 g/dL for other volatiles).

Result: During the fourteen-month period, the laboratory tested 1,818 DWI/DUID cases for alcohol. The mean/median BAC (range) was 0.177/0.180 (0.000-0.410) g/dL. The mean/median age was 35/33 (16-83) years; those aged ≥35 years had a significantly higher BAC than those aged <35 years (ANOVA, $P = <0.001$). Eighty percent were males but there was no significant difference in BAC between males and females ($P = 0.285$); 72% were White, 17% Black, 5% Asian, and 2% Hispanic. Four percent of the cases had BAC <0.050 g/dL, 5% between 0.050-0.079 g/dL, 4% between 0.080-0.099 g/dL, 19% between 0.100-0.149 g/dL, 34% between 0.150-0.199 g/dL, 23% between 0.200-0.249 g/dL, 9% between 0.250-0.299 g/dL, and 3% with ≥0.300 g/dL. The mean age of the BAC groups was 32-37 years except the ≥0.300 g/dL BAC group (40 years). The female proportion was higher in the 0.150-0.199 and ≥0.300 g/dL BAC groups (23%). The White proportion was significantly higher in the ≥0.300 g/dL BAC group.

Conclusion/Discussion: The mean BAC of the individuals involved in alcohol-impaired driving in Houston was comparable to the values (0.12-0.19) found in other studies (Jones and Andersson. J Forensic Sci 1996; Petković et al. Traffic Inj Prev 2016; Sun et al. Traffic Inj Prev 2014; Jones and Holmgren. Forensic Sci Int 2009). Impaired driving performance at BAC ≥0.050 g/dL has been demonstrated by numerous epidemiological, driving simulation, and laboratory studies. Our data revealed that reducing alcohol-impaired driving is a critical task for the Houston community. A number of the interventions targeting driving or drinking behaviors and related regulations have shown to be effective (Centers for Disease Control and Prevention MMWR 2015;64:814).

Keywords: Alcohol, Ethanol, Impaired Driving

S23

Opioid Trends in Palm Beach County, Florida from a DUID and Drug Seizure Perspective

Dustin Tate Yeatman, Nicholas Tiscione, Russell Miller, **Xiaoqin Shan***, Marcus Warner, Amber Kohl, Ilene Alford, Diana Lawrence, Steven Williams; Palm Beach County Sheriff's Office, West Palm Beach, FL

Background/Introduction: For many years, hundreds of pain clinics were located in South Florida dispensing millions of oxycodone and other addictive narcotic prescriptions every year. The term "pill mill" was coined for these clinics due to the relative ease with which individuals could obtain prescriptions for hundreds of highly addictive medications. Drug-seekers were obtaining multiple prescriptions a day while out of state drug traffickers were utilizing these clinics to supplement their supply. Various strategies at the local and state level were used to combat this issue including the implementation of the Florida Prescription Drug Monitoring Program (PDMP) in 2011.

Objective: The objective of this presentation is to describe trends in the prevalence of common opioids in specimens submitted to the Palm Beach County Sheriff's Office Toxicology and Chemistry Units and to examine the effect of the PDMP and other law enforcement efforts on prescription opioid abuse in South Florida in the past four years.

Method: The prevalence of the eight most common opioids identified in the Toxicology and Chemistry Units was tabulated on an annual basis from 2009 to 2016. The data was evaluated to identify trends in the popularity of these drugs over the seven year period and to determine the impact of the Florida PDMP and other law enforcement efforts.

Result: The most commonly identified opioids between 2009 and 2016 were fentanyl, heroin, hydrocodone, hydromorphone, methadone, morphine, oxycodone, and oxymorphone. Significant trends of these drugs in both Toxicology and Chemistry were observed including a significant increase in the prevalence of heroin and fentanyl with a substantial corresponding decline in the number of forensic samples in which oxycodone was detected. Seizures of heroin increased to 1315 cases in 2015which was ten times the number of seizures before the implementation of the PDMP while seizures of Fentanyl went from non-existent to routine detection in forensic samples. Detection of 6-monoacetylmorphine (heroin metabolite) and fentanyl in Toxicology samples also showed a significant increase during this time period.
Juxtaposed to the trend observed for heroin and fentanyl, seizures of oxycodone after implementation of the PDMP declined precipitously. By 2015, oxycodone seizures dropped by 81% compared to seizures in 2010. During the same period, detection of oxycodone in toxicology specimens decreased by nearly 73%.

Conclusion/Discussion: Data suggests that while the PDMP program, as well as other law enforcement efforts, were effective in stemming the flow of many prescription drugs in South Florida, a shift to opioids such as fentanyl and heroin by drug addicted individuals has given rise to another, possibly more serious, epidemic. Although the emergence of fentanyl was unexpected, the observed increase in heroin use was expected due to the reduced supply of prescription opioids available to opioid addicted individuals.

Keywords: Opioids, Trends, DUID

S24

Analysis and Detection of the Pyrolytic Products of JWH-018 and 12 Additional Synthetic Cannabinoids on 6 Species of Herbal Matrices

Stephen Raso*[1], Suzanne Bell[1, 2]; Departments of [1]Chemistry and [2]Forensic & Investigative Science, West Virginia University, Morgantown, WV

Background/Introduction: Synthetic cannabinoids have become a ubiquitous challenge in forensic toxicology and seized drug analysis. Synthetic cannabinoids are similar to Δ^9-tetrahydrocannabinol (THC) as they act as agonists at the cannabinoid receptors, but additional acute toxic effects are observed with their use including tachycardia, seizures, depression, possible suicidal tendencies, and psychotic episodes. The thermal degradation products have yet to be identified and evaluated for toxicity in comparison to parent and metabolic compounds. An investigation into these pyrolytic products, as the major route of ingestion is inhalation, may produce additional insight to understand the toxicity caused by synthetic cannabinoids.

Objective: To present detected thermal degradation products produced via the pyrolysis of JWH-018 and twelve additional synthetic cannabinoids to later be utilized in the evaluation of acute toxicity. The detected products described will provide additional target analytes in post mortem analysis. The products will be taken on for the analysis of cell toxicity. This knowledge could also be of use to the field of medicolegal death investigation and public health and safety.

Method: The pyrolysis of fourteen synthetic cannabinoids including AB-CHMINACA, AB-FUBINACA, AB-PINACA, AM-694, AM-2201, JWH-018, JWH-073, JWH-081, JWH-210, MAM-2201, PB-22, UR-144, and XLR-11 and six herbal plant products including *Althaea officinalis* (Marshmallow Leaf), *Combretum quadrangulare* (Sakae Naa), *Nepeta cataria* (Catnip), *Nymphaea caerulea* (Blue Lotus), *Turnera diffusa* (Damiana), and *Zornia latolia* (Maconha Brava) were carried out in a smoking simulator constructed of quartz glassware and a vacuum pump. The apparatus was optimized for temperature and vacuum pressure. The plant material was spiked with the drugs via solvent sprayer to achieve concentrations ranging from 2-5 % w/w. After pyrolysis, samples were collected from the glassware via methanol washes and the burnt residue was put through a methanolic extraction; each being filtered through 0.45µm cellulose acetate. The filtered samples were then evaporated under nitrogen gas, reconstituted in methanol, and analyzed on an Agilent 7890B-5977A gas chromatograph-mass spectrometer (GS/MS) with an HP-5 column.

Result: The pyrolysis apparatus was found to have the capability to reach adequate temperatures to induce pyrolysis of the herbal plant products and the vacuum pressure was optimized to a pressure of 6.77 kPa to simulate inhalation. The pyrolysis of the plant material alone was performed to provide a background for comparison between pyrolysis including drug compounds. The pyrolysis of the plant material alone produced 13 compounds that were consistently observed between the six different plant species. The products have been tentatively identified using the NIST Mass Spectrum Library with match scores of ≥80%, excluding one product whose score was 75%. After pyrolysis of each plant material with JWH-018, approximately 30 possible pyrolytic products are consistently observed and have been tentatively identified via library search. The pyrolysis of the remaining synthetic cannabinoids is part of ongoing work and will also be presented. Standards will be obtained to positively identify the pyrolytic products.

Conclusion/Discussion: The present study documents the presence of thermal degradation products of multiple synthetic cannabinoids via GC/MS analysis. The observed pyrolytic products are viable for analysis in post mortem samples and the evaluation of toxicity.

This work was supported by the National Institute of Justice [2015-R2-CX-0032].

Keywords: Synthetic Cannabinoids, Pyrolysis, Toxicity

S25
Synthetic Cathinone Stability in Urine Using LC/Q-TOF-MS

Lindsay Glicksberg*, Sarah Kerrigan; Department of Forensic Science, Sam Houston State University, Huntsville, TX

Background/Introduction: Synthetic cathinones are an emerging class of "designer drug" that produce psychostimulant-type effects. According to the National Forensic Laboratory Information System (NFLIS) cathinone abuse in the United States is increasing. Although most forensic laboratories can detect these drugs in seized materials, they are difficult to detect in biological evidence. Forensic laboratories must be able to identify these new drugs in living and deceased persons as part of antemortem and postmortem toxicology investigations. Anecdotally, some of the cathinones are reported to be unstable. It is important to understand drug stability in biological evidence in order to interpret analytical findings in criminal and death investigations.

Objective: To systematically evaluate the stability of twenty-two synthetic cathinones in urine using liquid-chromatography/quadrupole-time of flight-mass spectrometry (LC/Q-TOF-MS). Drug stability was assessed in terms of pH, temperature, and concentration-dependence.

Method: Solid phase extraction (CEREX Polycrom Clin II) and LC/Q-TOF-MS (Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS) equipped with a Poroshell 120 EC-C18 column were used to identify twenty-two synthetic cathinones in urine over a period of six months. Specimens were evaluated on the order of hours, days and weeks, depending on the rate of degradation. The following cathinones were included in the study: methcathinone, ethcathinone, pentedrone, buphedrone, 3-fluoromethcathinone (3-FMC), 4-fluoromethcathinone (4-FMC), 4-methylethcathinone (4-MEC), 4-ethylmethcathinone (4-EMC), mephedrone, methedrone, 3,4-dimethylmethcathinone (3,4-DMMC), ethylone, butylone, pentylone, eutylone, methylone, methylenedioxypyrovalerone (MDPV), 4-methylpyrrolidinobutiophenone (MPBP), 3,4-methylenedioxypyrrolidinobutiophenone (MDPBP), alpha-pyrrolidinopentiphenone (alpha-PVP), pyrovalerone, and naphyrone. A total of nine deuterated internal standards were used: methylone-d3, eutylone-d5, pentylone-d3, buytlone-d3, MDPV-d8, naphyrone-d5, mephedrone-d3, alpha-PVP-d8, and ethylone-d3. All twenty-two cathinones were separated in a run time of 13 minutes and the analytical procedure was validated according to the Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation. Limits of detection and quantitation ranged from 0.25 to 10 ng/mL. Cathinone stability was systematically evaluated at high (1000 ng/mL) and low (100 ng/mL) concentrations, at variable pH (pH 4 and 8) and at four temperatures (-20°C, 4°C, 20°C, and 32°C).

Result: The stability of synthetic cathinones were highly pH and temperature dependent. Moreover, chemical structure (ring substituted, unsubstituted, methylenedioxy-type, and pyrrolidine-type) profoundly influenced stability. Synthetic cathinones were more stable in acidified urine, with less than 40% loss in most cases even at the elevated (32°C) and room (20°C) temperatures over the 6 months. In alkaline urine (pH 8) there were significant losses for all drugs at elevated and room temperatures. In several instances, particularly with the ring-substituted cathinones, drugs were completely undetectable within twenty-four hours of storage. Under all conditions tested, the pyrrolidine-type cathinones were the relatively stable, with the methylenedioxy-pyrrolidine cathinones (MDPBP and MDPV) demonstrating the greatest stability. MDPBP and MDPV were stable at all temperatures in acidic urine, and at 4°C and -20°C in alkaline urine, over the entire 6-month period.

Conclusion/Discussion: Biological evidence may be subjected to a variety of environmental conditions prior to and during transport to the forensic laboratory. These findings demonstrate that some of the synthetic cathinones are inherently unstable. As a result, quantitative drug findings in criminal and death investigations should be interpreted cautiously, and within the context of specimen storage and disposition.

Keywords: Cathinones, LC/Q-TOF-MS, Stability

S26

Identifying Levorphanol Ingestion Using Urine Biomarkers in Healthcare Patients

Amber R. Watson[*1,2], Ali Roberts[1,2], Rebecca Heltsley[1], Timothy Robert[1], David L. Black[1,3]; [1]Aegis Sciences Corporation, Nashville, TN, [2]Belmont University College of Pharmacy, Nashville, TN, [3]Vanderbilt University Department of Pathology, Immunology, and Microbiology, Nashville, TN

Background/Introduction: Levorphanol is a long-acting opioid analgesic that binds mu, delta, and kappa opioid receptors; additionally, it decreases activity at N-methyl-D-aspartate (NMDA) receptors and blocks uptake of serotonin and norepinephrine. It is a chemical isomer of dextrorphan, which is a metabolite of the cough suppressant dextromethorphan. However, while dextromethorphan metabolizes to dextrorphan, 3-methoxymorphinan, and (+)-3-hydroxymorphinan, levorphanol metabolizes primarily to levorphanol-3-glucuronide and norlevorphanol, or (-)-3-hydroxymorphinan. Levorphanol was first marketed in the 1950s as an alternative to morphine for chronic cancer pain. Its use declined after the introduction of extended-release preparations of morphine, fentanyl, and oxycodone, and it is now only commercially available in the United States as a two milligram oral tablet. In more recent years, providers have expressed a renewed interest in utilizing levorphanol after two clinical trials examined its use for neuropathic pain, chronic pain, and in terminally ill cancer patients. As a result, there is an increased demand for levorphanol testing to assess treatment compliance.

Objective:

1. To investigate urinary concentrations of levorphanol/dextrorphan and 3-hydroxymorphinan.
2. To characterize cases of potential levorphanol ingestion based on detection of relevant urinary markers.
3. To assess the need for an isomeric analysis test method to distinguish between dextromethorphan and levorphanol ingestion.

Method: Urine test results for 279 samples submitted to Aegis between July 2014 and July 2015 were evaluated for the presence of levorphanol/dextrorphan and 3-hydroxymorphinan by liquid chromatography/tandem mass spectrometry (LC/MS/MS) above the limit of quantitation (LOQ) (10 ng/mL). An isomeric analysis was not performed; therefore, dextrorphan and levorphanol could not be differentiated. Urine specimens underwent hydrolysis with β-glucuronidase.

Result: *Per laboratory requisition form

Prescribed Medication*	*N*	Median Urinary Concentrations (ng/mL) [range]		Median Parent to Metabolite Ratio [range]
		Levorphanol/Dextrorphan	3-hydroxymorphinan	
Dextromethorphan	46	1,486 [14-35862]	1,121 [26-32,749]	1.4 [0.1-4]
Levorphanol	4	3031 [1997-12,070]	321 [173-1,241]	10.1 [8.3-11.4]
None Indicated	229	996 [0-325,789]	618 [0-237,625]	1.5 [0-21.9]
Total	**279**	**1133** [0-325,789]	**654** [0-237,625]	**1.5** [0-21.9]

Conclusion/Discussion: To our knowledge, this is the first report identifying urinary concentrations of levorphanol/dextrorphan and 3-hydroxymorphinan in human urine. Although metabolite concentrations would typically be expected to exceed parent concentrations in urine, the median parent to metabolite ratio for all samples was greater than 1 due to hydrolysis of glucuronide metabolites. Additionally, the LC/MS/MS method utilized to detect levorphanol/dextrorphan and 3-hydroxymorphinan does not distinguish between isomeric compounds; as a result, levorphanol/dextrorphan and (+)/(-)-3-methoxymorphinan could not be differentiated. Of the 46 samples with dextromethorphan listed as a prescribed medication, 16 (34.8%) did not have any detectable urinary concentrations of dextromethorphan or 3-methyoxymorphinan at the time of urine collection. Consequently, the potential exists for patients prescribed levorphanol to ingest dextromethorphan and appear compliant with levorphanol therapy on a urine drug test. Overall, it may be impossible to distinguish between levorphanol and dextromethorphan ingestion based on urinary markers, unless dextromethorphan or 3-methoxymorphinan are present or isomeric analysis is performed.

Keywords: Levorphanol, Dextrorphan, Urine Testing

S27

Comparison of Paired Umbilical Cord Tissue and Meconium Samples for Detection of *In Utero* Drug Exposure

Jennifer M. Colby*; Vanderbilt University Medical Center, Nashville, TN

Background/Objective: Prenatal exposure to pharmaceuticals, both illicit and prescribed, has many health consequences for neonates. One of the most severe is the development of neonatal abstinence syndrome, which can require weeks of treatment in a neonatal intensive care unit. Additional legal consequences exist for mothers, including temporary or permanent loss of custodial rights to the neonate. Drug-exposed neonates are often identified based on clinical presentation and history of maternal drug use, but exposure is typically confirmed via toxicology testing on specimens from the neonate. Meconium has long been considered the gold standard for neonatal drug testing, but practical considerations make it an unattractive specimen. Umbilical cord tissue has several advantages over meconium, including that it is available immediately after birth and requires a single collection, and limited evidence suggests that it may be used as an alternative specimen. The goal of this study was to compare the detection of drugs in paired meconium and umbilical cord tissue samples collected from babies born or treated within 5 days of birth at Vanderbilt University Medical Center between 10/1/13 and 4/8/15.

Method: Patients whose providers ordered both umbilical cord and meconium toxicology testing for clinical decision making and whose charts were available for review were included in this IRB-approved study. All toxicology testing was performed by a national reference laboratory (ARUP) using a combination of immunoassay and chromatography-mass spectrometry techniques. Additional details on analytical methods and limits of detection are available from ARUP at www.aruplab.com using test codes 92516 (meconium) and 2006621 (cord). Drugs that were only available in either the cord panel or the meconium panel were excluded from the analysis. Results were available for twenty-seven individual drugs belonging to one of 6 drug classes (amphetamines, cocaine, opioids, cannabinoids, benzodiazepines, barbiturates). Toxicology results were gathered from the laboratory information system and clinical information was collected through chart review.

Result: Paired umbilical cord tissue and meconium results were available for 217 neonates. Of these, 77 pairs were concordant negative and 36 pairs were concordant positive. The remaining 104 pairs of samples had at least one discordant result. When metabolites and parent drugs were grouped together, 66 of these became concordant. For the remaining 38 pairs, the results from cord indicated a different drug exposure than the results from meconium. The majority of these discrepancies were not explained by peri-natal drug administration. When considering drugs by class, the overall agreement between cord and meconium ranged from 75% (cannabinoids) to 100% (barbiturates). Considering meconium as the gold standard, cord was less sensitive than meconium for the detection of all drug classes except barbiturates. For 5 of the 6 individual drugs where adequate data were available (carboxy-THC, hydromorphone, oxycodone, oxymorphone, morphine), the concentration of drug measured in meconium did not correlate with qualitative detection in cord. For hydrocodone, a lack of detection in cord correlated with a low meconium concentration.

Conclusion/Discussion: Accurate drug detection and interpretation of toxicology results are important for identification and treatment of drug-exposed neonates. Toxicology results are also used to support any legal action that is taken to protect a drug-exposed neonate. This study demonstrates different sensitivities of drug detection in umbilical cord tissue and meconium, which indicates that these specimens are not completely interchangeable. These results can be used to help clinicians or toxicologists select the most appropriate test to confirm a suspected in utero drug exposure, or when testing is performed in multiple matrices, these results may help with the interpretation of discordant results. This study also highlights the need for additional research into the timing and mechanisms of drug deposition in umbilical cord tissue during pregnancy.

Keywords: Neonatal Abstinence Syndrome, Alternative Matrices, Drug of Abuse Testing

S28

Quantification of 11-Nor-9-Carboxy-THC in Hair Using a Hybrid Triple Quadrupole Linear Ion Trap Mass Spectrometer

Xiang He*[1], Adrian Taylor[2], Alexandre Wang[1]; [1]SCIEX, Redwood City, CA, [2]SCIEX, Concord, ON, Canada

Background/Introduction: A MS/MS/MS (MS^3) mass spectrometric method was developed on a hybrid triple quadrupole linear ion trap mass spectrometer (QTRAP) that reproducibly detects and quantifies the carboxy metabolite of Tetrahydrocannabinol (11-nor-9-Carboxy-Tetrahydrocannabinol, THC-COOH) in hair with a limit of quantification of 0.04 pg/mg.

Another method using the same mass spectrometer was evaluated that allows the identification and simultaneous quantification through MS/MS library matching and ion ratio value, for a panel of commonly analyzed drugs of forensic interest in hair covering amphetamines, opiates, cocaine and their metabolites.

Objective: Reproducibly detect and quantify the carboxy metabolite of Tetrahydrocannabinol (11-nor-9-Carboxy-Tetrahydrocannabinol, THC-COOH) in hair with a limit of quantification of < 0.05 pg/mg.

Method: Sample preparation for THC-COOH consisted of a methanol/water wash followed by a NaOH digest at 75 °C for one hour and then liquid/liquid extraction with hexane:ethyl acetate. Separate hair samples were spiked with the other compounds and an overnight acetonitrile/formic acid digest was used. HPLC separation used a reverse-phase column, 30 degrees centigrade, using a 8.0 minute gradient. A SCIEX 6500+ QTRAP system in MS^3mode was used for 11-nor-9-Carboxy-THC detection and Multiple Reaction Monitoring (monitoring 2 transitions per analyte), with Information Dependent Acquisition (IDA)-triggering enhanced product ion (MRM-IDA-EPI) scans for analysis of all other analytes

Result/Discussion: Detection of 11-nor-9-Carboxy-THC in hair by MRM is hampered by the complexity of the matrix and presence of compounds of similar structure and mass. This often leads to high background and interfering peaks in the MRM trace that does not allow the low detection requirements. In order to eliminate these interferences and background to improve the LOQ with good reproducibility, MS^2 fragmentation has the required selectivity to allow for the low level quantification. At the 0.2 pg/mg cutoff level in MRM mode the 11-nor-9- Carboxy-THC is hardly detectable in the high background and multiple interfering peaks but easily detectable using the MS^3 approach. THC-COOH levels in hair were quantified down to 0.04 pg/mg with 99% accuracy and less than 11% CV.
A separate scheduled MRM-IDA-EPI experiment was developed to detect and confirm the presence of parent and metabolite analytes from a targeted 22 compound panel in hair. The experiment allowed the simultaneous confirmation via MS/MS library matching (scores >75% for all compounds) and ion ratios (<20% CV for all analytes) as well as successful quantification of each compound in the panel with high precision (<10% CV for all analytes and at all concentration levels analyzed) was achieved by scheduling the MRM transitions and maximizing the time spent acquiring quality full scan MS/MS data to use in library searching. Linearity was from 5 pg/mg to 2.5 ng/mg for all compounds

Conclusion: A MS^3 method that reproducibly detects and quantifies 11-nor-9-Carboxy-THC levels in hair down to 0.04 pg/mg, was developed. A QTRAP method allowing confirmation for a panel of 22 forensic drug compounds was also developed.

Keywords: Mass Spectrometry, MS^3, THC-COOH

S29

Investigation into Applications of the NeoSAL™ Oral Fluid Collection Device for the Determination of Amphetamine and Methamphetamines

Ann-Sophie Korb*[1], Karen S. Scott[2], Fiona M. Wylie[1]; [1]Forensic Medicine and Science, University of Glasgow, Glasgow, Scotland, UK, [2]Forensic Science, Arcadia University, Glenside, PA

Background/Introduction: Oral fluid (OF) is no longer considered an alternative matrix, and is often collected when monitoring compliance with drug treatment programmes, in workplace and roadside drug testing. New guidelines for OF testing are constantly emerging, making it important to critically assess new collection devices. The newest collection device is the Neogen® NeoSAL™ device. It is a pad-based device that contains 2.1 mL of buffer, and is stated to collect 0.7 mL of oral fluid. No data on drug recoveries and applicability of the device exists and this is what this study aimed to change.

Objective: The objective of the study was to evaluate the applicability and advantages of the new Neogen® device for the analysis of amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxy-N-ethyl-amphetamine (MDEA) in OF samples. A partial validation was performed according to SWGTOX guidelines, collection volume adequacy and drug recoveries assessed, in accordance with cut-offs recommended by the European Workplace Drug Testing Society (EWDTS).

Method: Analytes were extracted using mixed-mode (UTC CleanScreen®) SPE followed by gas chromatrography-mass spectrometry (GCMS) for amphetamines in OF. The analysis used the respective deuterated compounds as internal standard (IS; 35 ng/0.7 mL of OF), over a calibration range of 2–200 ng/0.7 mL of OF. Samples were analysed in quadruplicate. A sample volume of approximately 2.8 mL (assuming 2.1 mL of buffer and 0.7 mL of OF) as per the manufacturer's specification was used. Validation parameters assessed included: linearity, limits of detection (LOD) and quantitation (LOQ), bias and precision (15, 30, 100 ng/0.7 mL of OF), interference, carryover, and processed-sample stability (15 and 100 ng/0.7 mL of OF). The device was assessed gravimetrically to establish collection volume adequacy as this is often overlooked in evaluation of collection devices. The drug recovery was assessed at two concentrations; 30 and 100 ng/0.7 mL of OF. Two methods were followed when assessing drug recoveries from the NeoSAL™ device: the collection pad was dipped into spiked OF and 0.7 mL of spiked OF was pipetted onto the pad.

Result: Calibration graphs were linear, showing r^2 values ≥0.999 (n = 10) for all curves. LOD was determined to be 0.71 ng/0.7 mL of OF (where S/N ≥3, n = 5); LOQ was set at 2 ng/0.7 mL of OF. Carryover was not seen for concentrations up to 1500 ng/0.7 mL of OF. None of the 40 drugs investigated showed interferences. Inter-day analytical accuracy (bias) and precision (n = 5) were 81–98%, and ≤3% for all three QC concentrations.
The analysis of processed-samples showed analytes were stable for up to 72 hours on the autosampler (19 ±0.5 °C), showing drug concentrations 67–105% and 84–97% of Day 0 concentrations at the two QCs over the testing period. Drug recoveries ranged from 63–81% for 30 ng/0.7 mL (n = 8), and 64–81% for 100 ng/0.7 mL (n = 8), which were higher than Intercept® i2™ recoveries (range 44–80%, n = 8) found in previous work. Gravimetric work (n = 25) carried out shows that the NeoSAL™ device collects an average of 0.84 mL (ranging from 0.61–1.1 mL, median 0.89 mL, %CV ≤15%) of OF compared to the stated 0.7 mL.

Conclusion/Discussion: Novel data was collected on the newest commercially available OF collection device. Although it is stated that the collection volume of the NeoSAL™ device is 0.7 mL, gravimetric work shows that OF can be collected in excess of the stated collection volume and drug recoveries for the amphetamine drugs from the device assessed were acceptable.

Keywords: Oral Fluid, Amphetamines, NeoSAL™

S30
Cocaine and Benzoylecgonine Oral Fluid On-Site Screening and Confirmation

Kayla N. Ellefsen*[1,2], Marta Concheiro,[1,3] Sandrine Pirard,[4] David A. Gorelick[5], Marilyn A. Huestis[1,6]; [1]Chemistry & Drug Metabolism Section, Clinical Pharmacology & Therapeutics Research Branch, IRP, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD, [2]Program in Toxicology, University of Maryland Baltimore, Baltimore, MD, [3]Department of Sciences, John Jay College of Criminal Justice, City University of New York, New York, NY, [4]University of Maryland Health Center, College Park, MD, [5]Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD, [6]University of Maryland School of Medicine, Baltimore, MD

Background/Introduction: Driving under the influence of drugs (DUID) is a major worldwide problem. Accurate on-site screening devices are necessary for rapidly identifying near the time of a traffic stop or incident if a drug is present in a driver. Oral fluid (OF) is a good biological matrix for on-site screening, as it can be collected non-invasively in a gender neutral manner at the time of driving; however, cocaine on-site OF screening device performance is variable. The Draeger DrugTest® 5000 is capable of detecting five drug classes including cocaine. Recently, Draeger developed a new benzoylecgonine (BE) test-strip with a 20 µg/L cutoff, with equivalent cross-reactivity to cocaine.

Objective: To evaluate for the first time OF detection windows and performance characteristics of the newly developed BE test-strip for the DrugTest 5000 and compare performance with the original platform for cocaine detection.

Method: Ten cocaine users provided OF, collected with the Draeger and either the Oral-Eze® or StatSure Saliva Sampler™ devices, up to 69 h following 25 mg intravenous (IV) cocaine. OF was first screened for cocaine and BE at 20 µg/L with the on-site Draeger DrugTest 5000. All positive and negative OF screening results were confirmed by a fully validated two-dimensional gas chromatography-mass spectrometry (2D-GC-MS) method for cocaine and BE, with evaluation of cocaine and BE test-strip performance at 1 (LOQ), 8 (SAMHSA), and 10 µg/L (DRUID) confirmation cutoffs. Additionally, detection rates and detection windows were determined by comparing screening results to cocaine and/or BE results at the same cutoffs

Result: All participants' OF screened cocaine and BE positive up to 2 h following cocaine IV administration, with the exception of one participant who screened positive for cocaine only at 1.5 h. Cocaine test-strip median T_{last} was 6.5 h for screening only results, and 6.5 h with Oral-Eze and 4 h with StatSure OF confirmation for cocaine and/or BE at 1, 8 and 10 µg/L. Sensitivity, specificity and efficiency ranged from 85.5-100% and 83.3-100% for cocaine only confirmation at 8 and 10 µg/L. For the BE test-strip, median T_{last} was 12.5 h for screening only and for cocaine and/or BE at all three confirmation cutoffs; sensitivity, specificity and efficiency ranged from 85.5-97.5% and 78.4-97.4% with cocaine and/or BE confirmation at 8 and 10 µg/L cutoffs, respectively.

Conclusion/Discussion: These are the first data to demonstrate that the DrugTest 5000 is a sensitive, specific, and efficient on-site OF screening device for cocaine and BE following controlled IV cocaine administration at confirmation cutoffs of 8 and 10 µg/L. The Draeger cocaine test-strip with cocaine only confirmation offers a useful option for monitoring the acute DUID intoxication phase; additionally, the BE test-strip with cocaine and/or BE confirmation increases the length of detection of cocaine intake for workplace drug testing, drug court, parole, pain management, and drug treatment programs and identified both the acute cocaine intoxication and cocaine crash/fatigue DUID phases.

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

Keywords: Cocaine, Benzoylecgonine, Draeger DrugTest 5000

S31

Detection Rates of Δ^9-THC, CBD, CBN, 11-OH-THC and THC-COOH in Hair Samples from Middle Eastern Cannabis Users Using Validated Methods for GC-EI-MS and 2D GC-NCI-MS

Farouq Alzahrani*[1], Fiona Wylie[1], Gail Cooper[2,3]; [1]Forensic Medicine and Science, School of Medicine, University of Glasgow, Scotland, [2]Office of Chief Medical Examiner, New York City, NY, [3]School of Medicine, University of Glasgow, Scotland

Background/Introduction: Cannabis is a commonly used illicit drug throughout the world, the active constituent being Δ9-tetrahydrocannabinol (Δ9-THC). The Society of Hair Testing (SoHT) in 2012 recommended 100 pg/mg of THC as a screening cut-off in hair and 0.2 pg/mg of THC-COOH as a confirmation cut-off.

Objective: The aims of this study were to develop and validate quantitative analytical procedures for the determination of Δ9-THC, cannabidiol (CBD), cannabinol (CBN), 11-hydroxy-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-Carboxy-THC (THC-COOH) in hair using GC-EI-MS and 2D GC-NCI-MS, and to apply these methods to the analysis of hair collected from addicts enrolled in a detoxification program in Saudia Arabia.

Method: Hair samples were collected from 20 cannabis users admitted to a detoxification ward in Al-Amal addiction hospital, Jeddah, Saudi Arabia. A 40-50 mg aliquot of sample was firstly washed using deionized water and then two times with dichloromethane (3mL of each solvent), for 3 min under sonication, and then dried overnight at room temperature. The sample preparation was based on an alkaline hydrolysis (1ml, 1M NaOH) of hair samples followed by liquid–liquid extraction (LLE) of cannabinoids under basic conditions using only 1.5 mL hexane/ethyl acetate mixture. The organic layer was then transferred into silanised high recovery vials and derivatised with 30 µl of BSTFA with 1% TMCS. To extract THC-COOH, the remaining LLE aqueous layer and/or Sample 'B' digest was acidified first using acetic acid and then introduced onto the hydrophobic/anion exchange solid-phase extraction (SPE) extraction cartridges. Eluent was then dried and derivatised with TFAA and HFIP. Agilent GC-EI-MS equipped with DB-5MS and 2D GC-NCI-MS equipped with an ultra-inert column DB-5MS, as a primary column, and HP-17MS as analytical column, were used for analysis.

Result: The method was validated and the results were satisfactory: bias within ±20% and within- and between-run precision below 12% and 18%. The LOQ for Δ9-THC (0.080 ng/mg) was below the cut-off value suggested by the Society of Hair Testing (SOHT) and 5 times higher for THC-COOH (1 pg/mg). Both methods demonstrated excellent linearity in the ranges 0.08–6.0 ng/mg, 0.2–6.0 ng/mg, 0.12–4.0 ng/mg, 0.16–4.0 ng/mg and 1.0-20.0 pg/mg, for Δ9-THC, CBD, CBN, 11-OH-THC and THC-COOH, respectively, with regression coefficients greater than 0.99.
Twelve (44.4%) of the 27 hair specimens had no detectable Δ9-THC, CBD, CBN, or THC-COOH at or above the described LOQs. THC-COOH was present in more hair specimens than Δ9-THC with 12 specimens (44.4%) having only THC-COOH, one (3.7%) only Δ9-THC and 3 (11.1%) with both. Δ9-THC was detected in 4 hair samples at concentrations ranging from 0.10 to 0.33 ng/mg (mean = 0.16, median 0.11). CBD had a high detection rate with 14 positive samples (51.8%) at concentrations ranging from 0.2 to 4.4 ng/mg (mean = 1.07, median 0.45). CBN was detected in 4 hair samples at concentrations ranging from 0.31 to 1.02 ng/mg (mean = 0.54, median 0.41) 11-OH-THC was not detected in the above described range. The main metabolite THC-COOH had the highest detection rate of all cannabinoids and was detected in 15 hair samples at concentrations ranging from 1.0 to 7.01 pg/mg (mean = 2.58, median 2.14).

Conclusion/Discussion: Significant lowering of limits of detection was possible to achieve by using the silanised high recovery vials and ultra-inert analytical column. CBD and THC-COOH have the highest detection rate.

Keywords: Cannabinoids, Hair, GCxGC-MS

S32
Ultra-Rapid Targeted Analysis of 63 Drugs in Hair by LC-MS/MS

Matthew Di Rago*[1,2], Tu Vo[1], Jessica Fernandez[1,2], Kerryn Crump[1], Voula Staikos[1], Linda Glowacki[1], Dimitri Gerostamoulos[12], [1]Victorian Institute of Forensic Medicine, Melbourne, Australia, [2]Department of Forensic Medicine, Monash University, Melbourne, Australia

Background/Introduction: Hair analysis provides many advantages over traditional specimen types. Hair sampling requires minimally invasive techniques for collection and can provide longer timeframes for drug detection when compared to other biological matrices. In some instances even single doses of drugs may be detected months to years after an alleged offence took place, depending on hair length. However, hair analysis can be limited by lengthy extraction techniques and chromatographic separations. Consequently, these methods require several days of processing, becoming time-consuming and costly.

Objective: The aim of this study was to develop a rapid hair analysis method that covered a broad range of drugs of abuse (including other impairing drugs), using reduced sample mass, a fast and simple extraction procedure and an improved LC-MS/MS analysis with automated data processing for rapid turn-around times.

Method: After washing, 20 mg of hair was homogenized in 1 mL of methanol over 12 minutes on an Omni™ BeadRuptor 24™ containing 2.8 mm ceramic beads. Following centrifugation for 5 minutes, the solvent layer was transferred to an autosampler vial and evaporated under a gentle stream of nitrogen gas. Extracts were reconstituted in 50 uL of methanol and 1 uL was injected into the LC-MS/MS system and analysed over 5 minutes. Sixty-three drugs of abuse and major metabolites were monitored including classes of; amphetamines, benzodiazepines, barbiturates, cocaine, opioids, cannabinoids and some novel psychoactive substances. Analytes were paired with six deuterated internal standards based on retention time (morphine-d3, MDMA-d5, buprenorphine-d4, diazepam-d5 and THC-d3 in positive mode and pentobarbitone-d5 in negative mode). Samples were separated using a Shimadzu Nexera X2 UPLC system with a C18 column (Kinetex C18, 4.6 × 50 mm, 2.6µm) using a gradient elution with a mobile phase of 50mM ammonium formate buffer (pH 3.5) / acetonitrile (0.5 min re-equilibration time). The drugs were detected using a Sciex™ API 4500 Q-TRAP™ LC-MS/MS system (ESI + and -, MRM mode, monitoring three transitions per analyte).

Result: The method was fully validated in accordance with international guidelines. Matrix effects and extraction efficiencies were acceptable with most analytes showing >80% response, with low variation (within 20% of mean response). Single samples of authentic cases were also repeatedly extracted to better estimate realistic extraction recovery, with primary extraction recovery ranging from 34-85%. The method was linear from 0.1-100ng/mL. Precision, accuracy and repeatability were satisfactory for all analytes investigated with relative standard deviations of approximately 8% or better in both drug-fortified (a batch of previously prepared hair incubated in a solution containing a high concentration of analyte to facilitate absorption into the matrix) and spiked hair samples (hair spiked with analyte at the time of extraction). The method was used to re-analyze 10 Quality Assurance Program cases (provided by the Society of Hair Testing) and results were compared to the programs' reported results as well as results from the laboratory's existing validated method (which utilized 18 hour sonication in methanol for analyte extraction). All previously reported analytes were detected including additional drugs and metabolites that were not included in the laboratory's previous method such as fentanyl, hydrocodone and hydromorphone. The method described has been successfully applied to the analysis of more than 50 hair specimens received as part of coronial and external fee-for-service forensic casework.

Conclusion/Discussion: The fast and reliable extraction method combined with rapid LC-MS/MS analysis of a broad range of drugs of abuse and automated data processing allows for the opportunity of greater throughput, reduced analysis cost and decreased turnaround times with the possibility of same-day analysis results.

Keywords: Drugs, Hair, LC-MS/MS

S33

Quantitative Determination of Drug Facilitated Sexual Assaults (DFSA) in Hair and Nails with Triggered Dynamic Multiple Reaction Monitoring (t-DMRM) With LCMSMS

Naga V. Naidu, Joseph A Cox, **Lauren Wolfe***, Adriana Trevino, Ernest D. Lykissa; Expertox, Deer Park, TX

Background/Introduction The range of drugs used in sexual assaults has increased significantly, resulting in a sizeable concern to the public. Laboratories encounter a wide range of substances in DFSA cases such as benzodiazepines, Z-drugs, muscle relaxants, hallucinogens and over the counter (OTC) drugs in addition to new classes of synthetic drugs like cathinones, LSD & NBOMes. In most cases, drugs used to facilitate these crimes are difficult to identify and detect due to amnesic properties associated at low doses, rapid renal clearance due to short half-life, and a delay in reporting such incidents by the victim(s). In these situations, blood and urine can be of limited use unless collection occurs proximal to the crime. Hair and nail testing is a suitable adjunct in conventional DFSA testing due to longer detection periods and repeatability of results. Due to the low concentrations of these drugs present in hair or nail specimens, a very sensitive quantification method is required. A new dynamic MRM method (triggered dMRM) introduced by the Agilent Technologies was employed to achieve the lowest concentrations[1].

Objective: Develop a simple and sensitive method for the measurement of substances used in DFSA in hair and nail samples.

Method: Forty-two drugs were selected as potential agents used in DFSA cases[2]. A simple acidified methanol extraction procedure was employed to extract drugs from samples[3]. Calibrators and controls were prepared in negative hair matrix obtained from Utak controls and treated like patient samples. Extracted samples and calibrators were evaporated and reconstituted with mobile phase. Analytes were separated on an Agilent UHPLC equipped with a Zorbax Eclipse C-18 (4.6x1.8x100) column with an isocratic elution of 0.1 % formic acid in water (60%) and 0.1% formic acid in acetonitrile at 0.5 mL/min flow rate for a run time of 7.5 mins. The t-DMRM method was developed and validated to analyze all drugs and internal standards (21). All analytes were monitored using multiple reaction mode with new triggered dynamic technology.

Result: Triggered DMRM technology allowed monitoring analytes during their time of elution only, which increased dwell time and resulted in more sensitivity. Patient samples (n=10) were also analyzed using the new method, and different classes of drugs were identified and quantified. All drugs analyzed established acceptable linearity ($\geq$0.995, n=6) between 10-500 pg/mg with a LOD of 5 pg/mg. No ion suppression was observed up to 2000 pg/mg.

Conclusion/Discussion: The new t-DMRM method developed is a sensitive, rapid and repeatable method to detect and quantitate DFSA in hair and nail samples.

References:

1) Triggered MRM: Simultaneous quantification and confirmation using Agilent Triple quadrupole LC/MS Systems. Technical Overview by Agilent Technologies, 2013.
2) Guidelines for the Forensic analysis of drugs facilitating sexual assault and other criminal acts: United Nations Office on Drugs and Crime, NY, 2011.
3) Guidelines for Testing Drugs under International Control in Hair, Sweat and Oral Fluid: United Nations Office on Drugs and Crime, NY, 2014.

Keywords: DFSA, Hair, Nails, Triggered Dynamic Multiple Reaction Monitoring

S34

Analysis of 37 Synthetic Cathinones and Other New Psychoactive Substances in Human Urine by Liquid Chromatography-Tandem Mass Spectrometry

Evelyn Goh*, Jye Ing Soah, Xin Yi Seah, Hooi Yan Moy, Yi Ju Yao, Chi Pang Lui; Analytical Toxicology Laboratory, Applied Sciences Group, Health Sciences Authority, Singapore

Background/Introduction: Synthetic cathinones, commonly known as "bath salts", are derivatives of cathinone, a beta-ketone amphetamine analogue found naturally in the Khat plant *(Catha edulis)*. These drugs are central nervous system stimulants, mimicking the effects of drugs such as amphetamine, methamphetamine and cocaine. In the recent years, the emerging trends of synthetic cathinones and other new psychoactive substances (NPSs) abuse such as phenethylamines, tryptamines and piperazines have been a worldwide issue. Toxicology laboratories are thus faced with the analytical challenge to detect and confirm these NPSs, some of which are present only in trace levels in biological samples. Therefore, it is important for the laboratory to develop a sensitive method for the simultaneous analysis of multiple classes of NPSs.

Objective: To develop and validate a confirmatory test method for the identification of 37 synthetic cathinones, phenethylamines, tryptamines, piperazines and other NPSs in human urine using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The analytes are listed in the table:

Cathinones
Cathinone
Buphedrone
Butylone
4-Chloro-2,5-dimethoxyamphetamine (DOC)
4-Chloromethcathinone (4-CMC)
Dimethylcathinone
3,4-Dimethylmethcathinone (3,4-DMMC)
Dimethylone (bk-MDDMA)
Ethcathinone
Ethylone
4-Ethylmethcathinone (4-EMC)
4-Fluoroethcathinone (4-FEC)
4-Fluoromethcathinone (4-FMC)
Mephedrone (4-MMC)
Methcathinone
4-Methoxy-α-pyrrolidinopropiophenone (MOPPP)
4-Methyl-α-pyrrolidinohexanophenone (MPHP)
4-Methyl-α-pyrrolidinopropiophenone (4-MePPP)
4-Methylbuphedrone
3,4-Methylenedioxypyrovalerone (MDPV)
4-Methylethcathinone (4-MEC)
Methylone
4-Methyl-α-pyrrolidinobutiophenone (MPBP)
Pentedrone
α-Pyrrolidinobutiophenone (α-PBP)
α-Pyrrolidinopentiophenone (α-PVP)

Phenethylamines
25B-NBOMe
25C-NBOMe
2C-I
Methiopropamine
4-Methoxymethamphetamine (PMMA)

Tryptamines
5-Methoxy-N,N-diisopropyltryptamine(5-MeO-DiPT)
5-Methoxy-N-methyl-N-isopropyltryptamine(5-MeO-MiPT)

Piperazines
1-Benzylpiperazine (BZP)
1-(3-Trifluoromethylphenyl)piperazine (TFMPP)

Other NPSs
Methoxetamine
Mitragynine

Method: During the sample preparation, 0.9 ml of urine sample was extracted using supported liquid extraction (SLE) method. Chromatographic separation was achieved on an ACQUITY UPLC HSS T3 (2.1x100 mm, 1.8 μm) column using gradient elution comprising of solvent (A) 10 mM ammonium formate in water and solvent (B) acetonitrile (0.1% formic acid), at a flow rate of 0.4 ml/min. Mass spectrometric data were acquired in multiple reaction monitoring (MRM) mode using positive electrospray ionization with three MRM transitions for each analyte. The analytical method was validated in accordance with the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines for qualitative methods. The parameters assessed included limit of detection, extraction recovery, matrix effects, carryover, stability and interferences. This validated method was applied to the analysis of urine samples obtained from suspected drug abusers.

Result: Using this method, all the 37 analytes were eluted within 7 minutes. The method was able to separate and specifically identify isobaric compounds, such as butylone and ethylone; 4-MEC and pentedrone. The limits of detection ranged from 0.5 to 5 ng/ml based on the requirements of three MRM transitions and their expected ion ratios. Extraction recoveries using SLE method were found to be in the range of 58% to 118%. Ion suppression and enhancement was assessed and shown to be between -18.1% to 24.6% (n=10). There was no evidence of carryover up to concentration of 2000 ng/ml. No significant endogenous interferences were observed, although, interferences from structurally-similar analytes and common drugs of abuse were observed for some of the analytes tested. Extracted samples were found to be stable for at least 48 hours when kept at 4°C. In the analysis of urine samples from suspected drug abusers in Singapore, ethylone, methylone, mephedrone, PMMA, methoxetamine and 5-MeO-MiPT have been detected, with ethylone being the most frequently detected NPS.

Conclusion/Discussion: A sensitive LC-MS/MS method for the detection and identification of 37 synthetic cathinones and other NPSs in urine had been successfully developed and validated. This validated method was successfully applied to the analysis of cathinones and other NPSs in authentic urine samples obtained from suspected drug abusers in Singapore.

Keywords: Cathinones, New Psychoactive Substances, LC-MS/MS Method Validation

S35

A Retrospective Study of Drug Facilitated Sexual Assault Cases in Lake County, Ohio

Amanda J. Jenkins*[1,2], Douglas E. Rohde[1]; [1]Lake County Crime Laboratory, Painesville, OH, [2]UMass Memorial Medical Center, Worcester, MA

Background/Introduction: The investigation of drug facilitated sexual assault [DFSA] cases may be limited due to lack of evidence. It is important that law enforcement personnel and hospital staff are thoroughly trained in toxicological specimen collection and appropriate history documentation. The forensic scientists at Lake County Crime Laboratory provide annual training for local law enforcement and sexual assault nurse examiners [SANE].

Objective: The objective of this study was to review all DFSA cases submitted by law enforcement during the period January 1, 2014 to December 31, 2015 in Lake County, Ohio for toxicology testing. Demographic information, police reports and laboratory results were collated.

Method: Typically, blood and urine were submitted for each case and a standard panel of tests conducted. These included volatiles by headspace gas chromatography in blood and urine; urine ELISA screen for amphetamine, barbiturates, benzodiazepines, cannabinoids, carisoprodol, cocaine/metabolites, fentanyl, flunitrazepam/metabolite, methadone, methamphetamine, opiates, oxycodone, phencyclidine, tramadol, tricyclic antidepressants and zolpidem; a general drug screen by solid phase extraction followed by gas chromatography-mass spectrometry for alkaline, neutral and acidic drugs and gamma hydroxybutyrate by gas chromatography-mass spectrometry. Additional confirmatory testing was performed as indicated by the case narrative and/or further investigation.

Result: A total of 20 cases were identified, ten for each year. Two of the cases were received from adjoining counties. The most common location of the incident was a residence [70%], followed by a vehicle [20%]. All but two alleged victims were female, with an age range of 4 to 46 years [N=14], with 71% of these individuals between the ages of 15 and 22. The majority of the incidents occurred between the hours of 2100-0500 and the alleged perpetrator was known to the victim in 18 cases, as an acquaintance [N=12], friend [N=5] or family member [N=1]. The majority of cases were reported to law enforcement within 12 h with only one case reported outside a 36 h window. The most common symptom reported was nausea/vomiting [N= 5] followed by drowsiness [N=4]. Drugs reported to be voluntarily ingested by the victims included ethanol [N=12, 60%], THC [N=4, 20%], and medications including citalopram, sertraline, tramadol [N=1], and valproic acid [N=1].

Blood and urine was submitted in 90% of the cases with one blood only and one urine only submission. More than one drug was identified in 12 cases. The most common drug detected was ethanol [N=9; 45%, blood 0.030-0.237 g%; urine 0.039-0.274 g%]. In 2 cases ethanol was detected in urine but not blood and 4 cases contained ethanol only. Other compounds reported included marijuana metabolite [N=8, 40%], benzodiazepines [N=3], cocaine metabolites [N=2], antihistamines [N=2], and acetone, amphetamine, codeine, sertraline/metabolite, dextromethorphan, citalopram, tramadol, fluoxetine/ metabolite, hydrocodone, ibuprofen, aripiprazole, topiramate, lamotrigine, lidocaine, and MDMA.

Conclusion/Discussion: Continued training of law enforcement and hospital staff in toxicological issues with DFSA cases resulted in successful compliance with timely, complete specimen collection. A two-year summation of DFSA cases revealed a demographic spread that included gender; an age range from pre-school to middle age; and a variety of detected drugs. Sustained communication during analysis and detailed explanation of confirmatory results were provided to investigators, however, none of the cases resulted in court proceedings.

Keywords: Drug Facilitated Sexual Assault [DFSA], Ethanol, Sexual Assault Nurse Examiner [SANE]

S36

On-site Oral Fluid Cannabinoid Screening After Controlled Smoked, Vaporized and Oral Cannabis Administration

Madeleine J. Swortwood[1], Matthew N. Newmeyer[1,2], Osama A. Abulseoud[1], Allan J. Barnes[1], Karl B. Scheidweiler[1], **Marilyn A. Huestis*[1,3]**; [1]Chemistry and Drug Metabolism, National Institute on Drug Abuse, Baltimore, MD, [2]Program in Toxicology, University of Maryland Baltimore, Baltimore, MD, [3]University of Maryland School of Medicine, Baltimore, MD

Background/Introduction: On-site oral fluid (OF) screening devices are increasingly popular for driving under the influence of drugs investigations. In addition to smoking, vaporization and edible consumption are common cannabis administration routes. Draeger DrugTest® 5000 (DDT5000) was previously evaluated after controlled smoked and vaporized cannabis administration; the Alere™ DDS®2 (DDS2) was not evaluated after controlled cannabis administration. Device performance evaluation with authentic OF after controlled cannabis administration is important for clinical and forensic toxicology drug testing programs.

Objective: Evaluate DDT5000 and DDS2 on-site OF screening device performance via comparison with paired confirmatory OF analyses following controlled smoked, vaporized and oral cannabis administration to frequent and occasional cannabis smokers.

Method: Eleven frequent (≥5x/week) and nine occasional (≥2x but <3x/week) cannabis smokers provided informed written consent for this Institutional Review Board-, FDA-, and DEA-approved study. The study was randomized, double-blind, and placebo-controlled with a crossover and double-dummy design. Over 4 sessions, participants were administered one active (6.9% Δ^9-tetrahydrocannabinol [THC]; 54mg) or placebo cannabis-containing brownie followed by one active or placebo cigarette or one active or placebo vaporized cannabis dose. Only one active dose was administered per session. OF was collected with the Quantisal™ confirmatory collection device followed by the DDT5000 (5µg/L THC cutoff) or DDS2 (25µg/L THC cutoff) on-site screening device before and up to 54 or 72h after dosing for occasional and frequent smokers, respectively. THC was quantified in Quantisal OF by liquid chromatography-tandem mass spectrometry with 0.2µg/L limit of quantification (LOQ). Suggested optimal performance criteria require ≥80% sensitivity (true positive [TP] screen tests divided by TP and false negative screen tests X100), specificity (true negative [TN] screen tests divided by TN and false positive screen tests X100), and efficiency (total true positive and true negative screen tests divided by total screen tests).

Result: Overall, 654 DDT5000 and 679 DDS2 results were paired with Quantisal confirmation results. At the much lower THC LOQ, sensitivity, specificity, and efficiency were 36.4, 98.8, and 52.8% for DDT5000 and 38.0, 97.9, and 54.6% for DDS2. At a 5µg/L THC confirmation cutoff, overall sensitivity, specificity, and efficiency were 81.4, 92.1, 89.3% for DDT5000 and 86.5, 92.8, and 91.2% for DDS2. Sensitivities with a 5µg/L THC confirmation cutoff after smoked, vaporized and oral cannabis were 76.3, 72.1, and 93.0% over the extended time course, respectively, for DDT5000 and 88.9, 79.1, and 82.9% for DDS2. Specificity and efficiency were ≥80% for frequent and occasional smokers for both devices after all cannabis routes with ≥5µg/L and ≥10µg/L THC confirmation cutoffs. Positive screening results utilizing a 5µg/L confirmatory cutoff after smoked, vaporized, and oral cannabis were detected up to 20, 12 and 20h, respectively, for DDT5000, and 20, 10 and 5h, respectively, for DDS2. However, at 5h post-dose, detection rates with a 5µg/L confirmatory cutoff after smoked, vaporized, and oral cannabis were 60, 20, and 40% for DDT5000 and 60, 30, and 20% for DDS2, respectively.

Conclusion/Discussion: For the first time, DDT5000 device performance was evaluated after controlled oral cannabis administration and DDS2 was evaluated after three controlled cannabis administration routes. Sensitivity, specificity, and efficiency were ≥80% for both devices after smoked, vaporized and oral cannabis when confirmed with a ≥5µg/L confirmatory THC cutoff.

Keywords: Oral Fluid, On-Site, Cannabinoids

S37
Don't Forget About Psilocin

Erin L. Karschner*, Sarah A. Shoemaker, Jeffrey D. Chmiel, Anastasia M. Berrier, Thomas Z. Bosy, Jeffrey P. Walterscheid; Division of Forensic Toxicology, Armed Forces Medical Examiner System, Dover AFB, DE

Background/Introduction: Psilocybin mushrooms have been ingested to produce hallucinogenic effects for thousands of years. Pharmacodynamic effects include impaired perception, ataxia, hyperkinesis, agitation, tachycardia, tremor, nausea and mydriasis. While pharmacological effects are similar to lysergic acid diethylamide, psilocybin is less potent and has a shorter duration of action. Psilocybin is dephosphorylated *in vivo* to form psilocin, a pharmacologically-active substituted tryptamine. Psilocin is primarily metabolized to psilocin-*O*-glucuronide by UDP-glucuronosyltransferase. Although case reports of psilocybin mushroom intoxication are limited, this presentation illustrates the need for appropriate screening and confirmation assays and describes the analytical challenges associated with their routine application.

Objective: To emphasize the importance of analyzing for psilocin in forensic casework.

Method: Blood and urine specimens from investigative cases were submitted for analysis. Samples were screened for psilocin by gas chromatography/mass spectrometry in full scan mode and were confirmed quantitatively in blood and qualitatively in urine by GC/MS in selected ion monitoring (SIM) mode. The limit of quantitation was 5 ng/mL.

Result: Since 2014, the Division of Forensic Toxicology has had 20 confirmed psilocin cases. Cases were submitted from Alaska, California, Kansas, New Jersey, Texas, as well as several other unnamed regions. Most subjects were male (11), while 7 were unidentified. Case histories often were undisclosed or vague; the most thoroughly documented cases are described:

Case 1: A 24-year-old male placed a call to 911, but when emergency personnel arrived, he was disoriented and denied calling for help. Toxicology analyses revealed psilocin present in the urine, but not in the blood.

Case 2: A 21-year-old male attracted police attention in response to a noise complaint, which escalated into a physical altercation upon police arrival. The individual's blood contained 5.2 ng/mL of psilocin, while the urine contained psilocin, as well as diphenhydramine.

Psilocin was not identified by a standard alkaline-extractable GC/MS screen in several cases, but was detected when samples were enzymatically hydrolyzed, extracted, derivatized and analyzed by GC/MS in full scan mode.

Conclusion/Discussion: Free psilocin may be present in blood and urine in low concentrations, but may not be detected in non-hydrolyzed basic drug screens, as a majority of total psilocin is conjugated to glucuronic acid. Unfortunately, commercial immunoassays are currently unavailable for high-throughput psilocin screening. Thus, sensitive and specific analytical techniques are required for psilocin analyses, particularly in cases where other suspected hallucinogens are ruled out.

Keywords: Psilocin, Hallucinogens, Hydrolysis

S38

Analysis of Valproic Acid, Salicylic Acid, and Ibuprofen in Whole Blood by GC-MS

Lisa T. Bain*, Jon B. Stephenson, Melanie L. Flater; Georgia Bureau of Investigation (GBI), Atlanta, GA

Background/Introduction: Valproic acid and salicylic acid are regularly requested compounds of interest at the Georgia Bureau of Investigation (GBI). Ibuprofen on an infrequent occasion will also be requested. While salicylic acid and ibuprofen are not always critical information for cause of death determinations, there still lies a need for testing in certain circumstances such as child fatalities. Valproic acid is required at our laboratory in a much broader range of cases from DUID to postmortem toxicology.

Objective: Several methods have been published analyzing these compounds, separately or in combination with other drugs. LC-MS/MS is implemented in many of the published methods, but valproic acid has only one multiple reaction monitoring (MRM) transition, providing little structural information. GC-MS offers more structural confirmation for valproic acid. Silylation was a technique previously utilized by the GBI, but due to the negative impact this derivatization has on GC-MS systems, butylation was investigated instead.

Method: Whole blood samples were processed using a solid phase extraction (SPE) method. The extraction began with the addition of 2 mL of 0.1 M phosphate buffer (pH 6) added to a 100 µL aliquot of whole blood that was fortified with internal standards mephobarbital (250 mg/L), valproic acid-d6 (250 mg/L), and salicylic acid-d4 (100 mg/L). Samples were applied to a UCT Clean Screen® ZSTHC020 mixed-mode column that was preconditioned with methanol, deionized water, and 0.1 M phosphate buffer (pH 6). After wash steps with 0.1 M phosphate buffer (pH 6) and 1N acetic acid, the column was air dried and the compounds of interest were eluted with methylene chloride. Following the derivatization step with a (1:1) 0.2M trimethyl ammonium hydroxide: DMSO mixture along with iodobutane, the compounds were extracted with ethyl acetate and analysis was performed by GC-MS using an Agilent ® 7890A GC interfaced with the 5975C Triple Axis MS Detector. The quant ions used for the method are 145, 120, and 161 for valproic acid, salicylic acid, and ibuprofen, respectively. The quant ions for the internal standards are 151, 124, and 274 for valproic acid-d6, salicylic acid-d4, and mephobarbital, respectively.

Result: The LOD and LOQ was determined to be 2 mg/L for salicylic acid, 1 mg/L for valproic acid, and 0.25 mg/L for ibuprofen. A seven point calibration curve with concentrations ranging from 20 mg/L to 120 mg/L was incorporated, and the average coefficient of determination (r^2) was 0.993 for valproic acid, 0.994 for salicylic acid, and 0.996 for ibuprofen. The percent recovery was determined to be 10% for salicylic acid, 81% for ibuprofen, and 58% for valproic acid. Samples were found to be stable after 24, 48, and 72 hours after initial analysis, following storage in two different environmental conditions (room temperature and freezer). Carry over was detected only in the negative control subsequent to the 600 mg/L sample, which contained approximately 2 mg/L of ibuprofen, 3 mg/L of salicylic acid, and no valproic acid. The variability and %CV for the compounds are described in Table 1.

Table 1. Variability and %CV for valproic acid, salicylic, and ibuprofen.

	Valproic Acid	**Salicylic Acid**	**Ibuprofen**
30 mg/L Variability	8%	5%	7%
60 mg/L Variability	7%	8%	11%
110 mg/L Variability	9%	7%	11%
30 mg/L %CV	3.60%	2.50%	3.50%
60 mg/L %CV	3.30%	3.60%	5.30%
110 mg/L %CV	4.20%	3.20%	5.20%

Conclusion/Discussion: The described method has established its capacity for the analysis of valproic acid, salicylic acid, and ibuprofen in whole blood. Analysis has also proven to be precise and accurate demonstrating variabilities of 7% for valproic acid, 8% for salicylic acid, and 11% for ibuprofen, with the greatest %CV being 5.3% for ibuprofen. This method also offers stable butyl derivatives of the analytes in a clean solvent for GC/MS analysis that is less detrimental to instruments than silylation procedures, making it capable of replacing the GBI's previously used method.

Keywords: Acid Compounds, GC-MS, Butylation

S39
Monitoring Synthetic Cannabinoid Usage in Washington, DC

Samantha Tolliver*[1], Kenan Zamore[2], Sophia Brathwaite[1], Lucas Zarwell[1], Roger Mitchell[1]; [1]Washington, DC Office of the Chief Medical Examiner, Washington, DC, [2]Washington, DC Department of Health, Washington, DC

Background/Introduction: In 2015 Washington, DC Fire and Emergency Management Services (FEMS) identified an increase in transports due to synthetic cannabinoids usage. As in many jurisdictions, hospitals in the Washington, DC area do not have the ability to test for synthetic cannabinoids. Thus the data relating to the calls was largely reliant upon self-reporting or witness statements. From January through mid-May 2015, FEMS averaged five calls per day related to synthetic cannabinoids. However, through internal call tracking, FEMS noted that from mid-May through early July, the number of calls for synthetic cannabinoids increased to average 16 transports per day. As a result of the increase, an inter-agency collaboration between the Washington, DC Department of Health, FEMS and the Office of the Chief Medical Examiner (OCME) formed in order to develop and implement a hospital surveillance program. The surveillance began in mid-July 2015 and continues to date. Between the months of July – September 2015, the total number of synthetic cannabinoid related transports surged to an average of 32 per day. The role of the OCME toxicology laboratory was to facilitate the testing of all collected samples.

Objective: The objectives of this surveillance project were to confirm the surge in FEMS requests were related to synthetic cannabinoids. As well as, collect analytical data to reflect the usage and identify the synthetic analogs present within the Washington, DC area.

Method: The emergency departments from ten area hospitals were given instruction to ensure the collection of urine or blood, as well as demographic information (gender, race and age) from any emergency department patient suspected to be under the influence of synthetic cannabinoids. Those samples were stored in refrigeration (2 – 8 °C) and transported to the OCME Toxicology laboratory.

The laboratory accessioned, documented the demographic information and prepared each case to be sent to a reference laboratory for testing. The testing scheme included qualitative identification of synthetic cannabinoids, which were both screened and confirmed by liquid chromatography/ mass spectrometry/ mass spectrometry (LC/MS/MS). In addition, an immunoassay screen for drugs of abuse (DOA) followed by confirmation and quantitation by gas chromatograph/ mass spectrometry (cocaine and metabolites) and LC/MS/MS (all other analytes). Included within the urine DOA screen were amphetamines, cocaine and metabolites, methamphetamine, opiates, oxycodone/ oxymorphone and phencyclidine. The blood sample screen was inclusive of the urine analytes, as well as methylenedioxymethamphetamine.

Result: Between July 2015 and February 24, 2016, four hundred and sixty-five cases were submitted for testing. The testing population was largely comprised of African American males with a mean age of 35.8 years old. In the entire sampling population 26.4% tested positive for drugs of abuse (DOA) only, 26.9% tested positive for synthetics only, 29.7% tested positive for both DOA and synthetics. Among the synthetics results, the most commonly encountered urine metabolites were AB-Chminaca 3-methyl butanoic acid (31.5%), 5-fluoro PB-22 3-carboxyindole (21.9%), AB-Pinaca N-pentanoic acid (14.2%), UR-144 N-pentanoic acid (9.3%) and ADB-Chminaca (7.6%). Within that population, when DOA were detected marijuana, phencyclidine (PCP), cocaine and morphine/heroin with percentages of 35.0%, 26.7%, 22.9% and 11.5%, respectively, were the most frequently encountered. Additionally, twenty-two percent of the samples submitted were blood. The most commonly detected parent drugs were ADB-Chminaca (19%), ADB-Fubinaca (10.3%) and AB-Chminaca (9.0%).

Conclusion/Discussion: While the use of DOA has a continued presence in the District of Columbia, based on the analytical data, the surge in FEMS calls was in fact linked to synthetic cannabinoids. The inter-agency collaboration proved to be an effective way to help assess the needs of the community, as well as identify specific analogs present there within.

Keywords: Synthetic Cannabinoids, Surveillance Testing and Toxicology Findings

S40

The Ever-Changing Scope of Synthetic Cannabinoids in Toxicology Casework (2011-2015)

Kevin G. Shanks*, George Behonick; Axis Forensic Toxicology, Indianapolis, IN

Background/Introduction: Synthetic cannabinoids are laboratory developed chemicals that bind to cannabinoid receptors 1 and 2 in the human body. Many of the compounds have origins in academic research, while others have no formal history. In the USA, synthetic cannabinoids have been sold as ingredients in herbal incense, herbal potpourri, or smoking blends for the last several years. Since 2011, the Federal government has passed various waves of legislation classifying several synthetic cannabinoids as Schedule I controlled substances, i.e. substances considered to have a high potential for abuse, a potential for severe psychological or physical dependence, and have no currently accepted medical use in the USA. These substances are illegal to possess, manufacture, and distribute. As this legislation is enacted, manufacturers and vendors of these substances and resulting mass-produced retail products vary the active ingredient(s) to now uncontrolled substances. Currently there are 25 synthetic cannabinoids explicitly controlled by the Federal government as Schedule I controlled substances. AIT Laboratories/Axis Forensic Toxicology started testing for synthetic cannabinoids in human performance and postmortem blood toxicology casework in 2010. The scope of analysis has increased over time from 2 analytes to 51 analytes.

Objective: Attendees of this presentation will learn about the changing scope of synthetic cannabinoids in toxicology casework over a 5 year time period (2011-2015). A year by year depiction of the overall trends in scope of synthetic cannabinoids detected will be presented.

Method: All blood specimens were prepared via a liquid-liquid extraction into either ethyl acetate:hexane (98:2) or ethyl acetate. Instrumental analysis was completed by liquid chromatography with tandem mass spectrometry (LC/MS/MS) on a Waters Acquity UPLC coupled to a Waters Quattro Premier XE tandem quadrupole mass spectrometer. Method validation was carried out according to in-house method validation standard operating procedures and protocols. Attributes assessed during method validation included linearity, accuracy and imprecision, exogenous drug interferences, carryover, and ion suppression/matrix selectivity. A post-hoc review of toxicology results in the laboratory information management system (LIMS) was completed and data was tabulated.

Result: During the 5 year time period evaluated, the positivity rate for synthetic cannabinoids in analyzed blood casework was 23.2%. A total of 33 different synthetic cannabinoids were detected across the time range with a large increase in breadth of compounds in 2014-2015. In 2011, JWH-018 was the most prevalent compound detected (90%). In 2012, AM-2201 was the most prevalent compound (24%), while 2013's most prevalent compound was 5F-PB-22 (48%). In 2014 and 2015, AB-CHMINACA was the most detected compound in casework (45% and 33% respectively). A correlation between pending/passed legislation controlling compounds and synthetic cannabinoids appearing or disappearing in casework was observed.

Conclusion/Discussion: As compounds are controlled or scheduled by the government, manufacturers and vendors of synthetic cannabinoid containing products switch to newer uncontrolled substances, which in turn can be seen in toxicological casework. The number of synthetic cannabinoid compounds detected in blood casework has increased over the last five years with the largest increases realized over the last two years.

Keywords: Synthetic Cannabinoids, Trends, Toxicology

S41

Evaluation of the Nicotine Particle Size in an Aerosol Formed by an Electronic Cigarette

Jesse L. Patterson*[1], Justin L. Poklis[2], Joseph W. Stone[1], Michael Hindle[3], Joseph B McGee Turner[4], Alphonse Poklis[1,2,5], Michelle R. Peace[1]; [1]Department of Forensic Science, [2]Department of Pharmacology & Toxicology, [3]Department of Pharmaceutics, [4]Department of Chemistry, [5]Department of Pathology, Virginia Commonwealth University, Richmond, VA

Background/Introduction: Electronic cigarettes have become readily available as drug delivery systems. They use an e-liquid primarily composed of the humectants propylene glycol (PG) and/or vegetable glycerin (VG) and an active ingredient, often a drug, such as nicotine. An aerosol is produced by the electronic cigarette when the e-liquid passes over the heated coil, vaporizes, and then condenses with water in the atmosphere. The size of the droplets formed in the aerosol can vary. The size of the particle is a major factor in determining where and if that particle will deposit in the lung. Small particles can penetrate deeper into the alveoli of the lung tissue and increase their probability of being absorbed in to the blood stream.

Objective: To evaluate whether or not a typical electronic cigarette, KangerTech AeroTank, 1.8Ω preassembled atomizer, and an eGo-V2 at variable voltage battery, is capable of producing an aerosol with a significantly small enough particle of an active drug, nicotine, to be deposited it the lung for absorption in to the blood stream.

Method: A 12 mg/mL nicotine 50:50 PG:VG e-liquid formulation was aerosolized 10 seconds into a 10-stage Micro-Orifice Uniform Deposit Impactor™ or MOUDI (MSP, Corp., Minneapolis, MN) at a flow rate of 30 L/min. Each stage of the MOUDI represented a different particle size range, from 0.05 to 18 µm. The concentration of nicotine on each stage of the MOUDI was determined using an Applied Bio systems 3200 Q trap with a turbo V source for TurbolonSpray (Carlsbad, CA) with a Shimadzu SCL HPLC system (Shimadzu, Kyoto, Japan). Chromatographic separation was performed on a Hypersil Gold 3mmx50mm, 5 µm column (Thermo Scientific, Waltham, MA). The injection volume was 10 µL with a flow rate of 0.5 mL/min. The ionspray voltage was set to 5000 V with a declustering potential of 35 eV and the source temperature was 600 °C with 30 mL/min curtain gas flow. Ion source gasses 1 and 2 were set to 50 mL/min and 30 mL/min, respectively. The total run time for this method is 2 minutes and the instrument was operated in multiple reaction monitoring mode (MRM) monitoring the following *m/z* transitions: nicotine, 163>130 and 163>117; and nicotine-d4, 167>134. This experiment was performed at three different voltages of 3.9, 4.3, and 4.7 V on the electronic cigarette.

Result: The percent of recovered nicotine was determined for each stage. Nicotine concentrations were below the limit of detection on stages 1-6 (18 to 0.54 µm) for 3.9 V and 4.7 V and stages 1-3 at 4.3V. Only 15% of the aerosol was collected on stages 4-6 (3.2 to 0.54 µm) at 4.3 V, leaving most of the aerosol in the remaining stages. Stages 7 and 8, representing particle size ranges of 0.54 to 0.31 µm and 0.31 to 0.172 µm respectively, consistently collected approximately 33% of the aerosol. The 3.9 V produced an aerosol with more particle sizes >10 µm. Additionally, all 3 voltages produced ultrafine particle sizes, <0.1 µm.

Conclusion/Discussion: Compared to traditional cigarette smoke, the aerosol produced by e-cigs have similar particle size production, centered at 0.3 µm (±), but also produced more ultrafine particles than a traditional cigarette. Therefore this type of electronic cigarette is capable of producing small enough particles of an active drug, nicotine, to be deposited it the lung for absorption in to the blood stream.

Keywords: Electronic Cigarette, Nicotine, Particle Size

S42

Novel High-Sensitivity Analysis of Cannabinoids from Whole Blood by Combining a HybridSPE Method and LC/MS/MS

Xin Xu*, Eric S. Lavins, Szabolcs Sofalvi, Harold E. Schueler, Thomas P. Gilson**;** Cuyahoga County Medical Examiner's Office (CCMEO), Cuyahoga County Regional Forensic Science Laboratory (CCRFSL), Toxicology Department, Cleveland, OH

Background/Introduction: Marijuana is the most commonly abused drug in the United States. It can cause behavioral effects such as euphoria, relaxation and mood changes. Delta-9-tetrahydrocannabinol (Δ^9-THC) is the main psychoactive ingredient in marijuana which is metabolized to active 11-hydroxy-delta-9-THC (11-OH-Δ^9-THC) and inactive 11-nor-9-carboxy-delta-9-THC (Δ^9-THC-COOH) in the body. Blood cannabinoid concentrations can be as low as nanogram to subnanogram (picogram) per milliliter levels after cannabis administration. Efficient sample extraction and highly sensitive quantification methods for the analysis of cannabinoids in biological specimens are desired to improve workflow, case analysis and interpretation of the results in postmortem death investigations and driving under the influence of drugs (DUID) cases.

Objective: To develop a quantification method for the analysis of Δ^9-THC and the metabolites from whole blood by combining efficient HybridSPE sample extraction and high sensitivity LC/MS/MS detection.

Method: The first step was to remove the proteins from whole blood using a Multi-Tube Vortexer (Scientific Industries, Inc.) and drop-wise addition of cold acetonitrile. The collected supernatant was then purified via HybridSPE columns (Sigma Aldrich, Inc.) to remove the phospholipids. The collected eluent was evaporated to dryness and reconstituted in mobile phase for LC/MS/MS analysis. Separation was performed on a C18 HPLC column (Thermo Scientific, 100 mm x 2.1 mm; 3 μm) with a mobile phase comprised of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). MS quantification was achieved using positive electrospray ionization (ESI) and selective reaction monitoring (SRM) with a TSQ Vantage triple quadrupole system (Thermo Scientific, Inc.).

Result: The first step of protein precipitation for the THC blood specimen usually involves vortexing individual samples while adding cold organic solvent which is labor-intensive and time-consuming. In this work, a Multi-Tube Vortexer was applied to automate the vortexing process for high throughput which significantly reduced the manual labor and preparation time. The protein-free supernatant was then processed with a novel HybridSPE procedure using a stationary phase of Zirconia-coated silica which efficiently removed the interfering phospholipids from the sample matrices. The quantification method using LC/MS/MS exhibited very low limits of quantitation: 0.2 ng/mL for Δ^9-THC; and 0.4 ng/mL for 11-OH-Δ^9-THC and Δ^9-THC-COOH. The linear ranges were 0.2 - 20 ng/mL for Δ^9-THC and 0.4 - 40 ng/mL for both of the metabolites with $r^2 > 0.99$. The recoveries for each drug were between 60 to 80% and ion suppressions were less than 15%.

Conclusion/Discussion: A highly sensitive and fast method was developed for the quantification of Δ^9-THC and the major metabolites from whole blood by combining a novel extraction procedure with LC/MS/MS detection. The efficient extraction procedure involved the use of Multi-Tube Vortexer for high-throughput processing and HybridSPE for the removal of phospholipids for improved sensitivity. This method can be applied to the forensic toxicology analysis of cannabinoids in postmortem death investigations and DUID cases.

Keywords: Cannabinoids, HybridSPE, LC/MS/MS

S43
Detection of Biomarkers in Heroin Abusers' Urine in Singapore

Joo Chin Tan*, Saw Leng Koh, Hooi Yan Moy, Yi Ju Yao, Chi Pang Lui; Health Sciences Authority, Singapore

Background/Introduction: Heroin is one of the prevailing drugs of abuse in Singapore besides methamphetamine. Currently, the laboratory analyses morphine, codeine and 6-monoacetylmorphine (6-AM) in urine samples of suspected drug abusers for the detection of heroin abuse. 6-AM is widely accepted as a specific marker of heroin abuse. However, due to its short detection window, 6-AM is detectable only in some of the urine samples from heroin users. Hence it is of interest to the laboratory to look for other compounds as indicators for heroin abuse.

Objective: To develop a method for the detection and identification of heroin biomarkers or correlative compounds in urine using liquid chromatography tandem mass spectrometry (LC-MS/MS). Besides morphine, codeine and 6-AM, other heroin biomarkers and heroin associative analytes included in this study are diamorphine, acetylcodeine, meconin, thebaine and chloroquine. Chloroquine is an anti-malarial drug but it is also a common additive present in the heroin abused by our local users.

Method: Urine samples obtained from suspected heroin abusers were screened using Roche Online DAT Opiates II urine drug screening assay. Subsequently, the positively screened urine samples were subjected to liquid-liquid extraction (LLE) under basic condition using ethyl acetate, followed by instrumental analysis on a Waters Acquity UPLC coupled to a XEVO TQD tandem Mass Spectrometer. Deuterated 6-AM (D_6-6-AM) was used as the internal standard. Separation was achieved using an Acquity BEH C18 (2.1 x 100 mm, 1.7 um) column by gradient elution with 10 mM ammonium formate containing 0.1% formic acid as solvent A and acetonitrile containing 0.1% formic acid as solvent B, at a flow rate of 0.4 ml/min. The analytes examined (diamorphine, morphine, codeine, 6-AM, acetylcodeine, meconin, thebaine and chloroquine) were efficiently chromatographed between 2.0 to 5.0 min. Multiple reaction monitoring (MRM) under positive polarity with ~~3~~ three transitions for each analyte was used. Total morphine and codeine levels in the urine were determined using an existing validated method on an Agilent HP6890+ series coupled to a 5973N gas chromatography-mass spectrometry (GC-MS) system fitted with a HP-5 column (12.5 m x 0.2 mm i.d., 0.33 μm).

Result: Validation data for LC-MS/MS method were attained for limit of detection, carryover and interferences. The detection limits for all the analytes were found to be at least 1 ng/ml except chloroquine which has a detection limit of 50 ng/ml due to its poor peak shape under the chromatographic condition. No carryover was observed up to 250 ng/ml for meconin, up to 500 ng/ml for acetylcodeine, thebaine and diamorphine, and up to 3000 ng/ml for chloroquine. No matrix interferences with any of the analytes were observed from the study of 10 different blank urine samples. Interferences from D_6-6-AM and other commonly encountered drug analytes were also found to be absent. A total of 276 suspected heroin abusers' urine samples were analyzed. Diamorphine was not detected in any of the urine samples. Of the 276 samples, 100 were found to be positive for 6-AM, in which 50 were also positive for acetylcodeine, and 2 were found to also contain meconin. Chloroquine was also detected in 49 of the 100 6-AM positive samples. The remaining 176 samples which were negative for 6-AM, all were found to be positive for both morphine and codeine, of which 59 samples were found to contain chloroquine, 4 samples contained acetylcodeine, and 2 samples contained thebaine. It is also interesting to note that out of the 59 samples where chloroquine was present, 30 samples contained high levels of morphine relative to that of codeine, which is consistent with heroin consumption.

Conclusion/Discussion: The data demonstrate that 6-AM is a good biomarker of heroin abuse as compared to acetylcodeine (100 6-AM positive samples versus 54 acetylcodeine positive samples). The detection of meconin and thebaine were much less frequent in the urine samples surveyed, possibly due to the absence of noscapine and thebaine in the heroin prevailing in the local illicit drug scene. The results also suggest that the presence of chloroquine correlates well with heroin use when morphine is detected in high levels in the urine samples with the absence of 6-AM.

Keywords: Opiates Biomarkers, Acetylcodeine and 6-Monoacetylmorphine

S44

Development of Statistical Tools for SWGTOX Method Validation of 11 Benzodiazepines in Whole Blood by SPE and GC/MS

Szabolcs Sofalvi*, Harold E. Schueler, Xin Xu, Claire K. Kaspar, Cassandra L. Clyde, Eric S. Lavins, Thomas P. Gilson; Cuyahoga County Regional Forensic Science Laboratory, Toxicology Department, Cuyahoga County Medical Examiner's Office (CCMEO), Cleveland, OH

Background/Introduction: As more laboratories become accredited by ABFT and ASCLD/LAB-*I*, continuously improving procedures is necessary in order to comply with the evolving standards of each accrediting agency. One of the industry standards in applied analytical chemistry is chromatographic quality. Chromatographic quality is important in both drug-facilitated sexual assault (DFSA) and driving-related impairment (DUI/OVI) cases when the results are above the limit of detection (LOD) but below the limit of quantitation (LOQ). Chromatographic quality is measured by various parameters such as the tailing factor (TF) with acceptable values being less than 2. The benzodiazepine procedure at the CCMEO was optimized so that all 11 analytes met this requirement. The 2013 SWGTOX Standard Practices for Method Validation were applied during the validation of this method and resulted in a plethora of data which required statistical analyses and processing.

Objective: The first objective of this work was to develop an analyte-specific validation template in Microsoft Excel which would minimize data entry and not require repeatedly performing regressions and one-way analysis of variance (ANOVA) calculations whenever the source data (concentration of the analyte or response ratio) changed. The second goal was to demonstrate the applicability of this template using the validated benzodiazepine method.

Method: The designed Excel template includes the following tabs: *Validation Summary Report*, *Calibration Model*, *Residual Plot*, *LOD*, *Bias and Precision*, *LOQ*, *Interference Studies*, *Carryover*, *Recovery*, *Dilution Integrity*, and *Stability*. The drug and internal standard responses from five trials were entered into the *Calibration Model* tab. The estimated *LOD* was calculated by the template using the equation $= \frac{3.3\ \sigma_y}{slope_{avg}}$. Bias and precision data were generated by Excel after entering 15 quality control results from triplicate runs of five different batches. Likewise, within-run and between-run coefficient of variation (%CV) for *LOQs* were also obtained in the *LOQ* tab. Percent recoveries and dilution integrities were obtained similarly. The contents of the ANOVA including the *Residual Output* were also calculated by the template automatically.

Result: The *Validation Summary Report* was prepared automatically by the template referencing the corresponding data located in different tabs. The *Calibration Model* used linear regression with a 1/x weighting factor. Based on the *Residual Plot*, the random distribution of individual residuals around the zero line suggested that a linear model was appropriate for all 11 analytes. Linearity was established from 6 to 100 ng/mL for lorazepam and alpha-hydroxymidazolam; 10 to 400 ng/mL for clonazepam, midazolam, alprazolam, alpha-hydroxyalprazolam, and temazepam; and 20 to 800 ng/mL for diazepam, oxazepam, and nordiazepam with $R^2 > 0.99$. 7-aminoclonazepam met qualitative acceptance criteria only. Within-run and between-run %CV of the low and high quality controls were between 2 and 20% (n=15). The average bias ranged between 5 and 10% from the theoretical targets. All analytes had a tailing factor value less than 2.

Conclusion/Discussion: The advantage of the customized statistical tool for Excel is to complete a method validation fast, accurately and without having to perform repeated statistical calculations which involved reformatting the page every time. Each analyte required five ANOVA tables for the *Residual Plot* tab, two for the *Bias and Precision* tab, and one for the *LOQ* tab. This corresponded to eighty eight total ANOVA tables required to complete the validation for all eleven analytes. The statistical tool was successfully applied to the benzodiazepine procedure at the CCMEO. This template provides a fast and efficient means to perform a multitude of statistical calculations for all assay validations as well and can be beneficial for other toxicology laboratories nationwide.

Keywords: Statistics, ANOVA, Method Validation, Tailing Factor

S45

Identification of Metabolites of U47700 (an Emerging Designer Opiate) from a Toxic Exposure

Anna Miller[1], Melissa Goggin[1], An Nguyen[1], **Gregory C. Janis*[1]**, Matthew Jones[2], Bradley Hernandez[2], Samuel Stellpflug[2]; [1]MEDTOX Laboratories, Laboratory Corporation of America® Holdings, [2]Regions Hospital, Saint Paul, MN

Background/Introduction: U47700 is a synthetic opiate patented in 1976 by The Upjohn Company. U47700 is one of a small family of esoteric opiate agonists lacking significant structural similarity to any other opiate drug class. None of these opiates were ever marketed or researched extensively. In the last year U47700 has surfaced as a designer drug without apparent legal restriction in the USA. Recently we were presented with a suspected U47700 overdose.

Objective: We set up to explore urine and serum samples collected from this case to determine the metabolism of U47700 for the purpose of defining optimal markers for toxicological testing detecting U47700 exposures.

Method: Urine and serum samples were initially analyzed using an untargeted liquid chromatography mass spectrometry (UPLC-MS) procedure. Sample aliquots were processed with and without enzymatic hydrolysis, and subsequently clarified. Sample supernatants were analyzed on a UPLC-time-of-flight mass spectrometer (Waters ACQUITY UPLC® and Waters Xevo® TOF mass spectrometer) using a slow ammonium acetate / acetonitrile gradient through a HSS C18 column. Full spectrum, high resolution data with and without collision induced fragmentation was collected. The data was mined for the presence of U47700 and its metabolites, as well as related designer opiates, other opiates, drugs of abuse, and non-opiate pharmaceuticals. Once metabolites were qualitatively identified, the samples were subjected to an independent quantitative analysis. Clarified samples were analyzed via a UPLC MS/MS procedure on a Waters ACQUITY UHPLC® system (Waters Corporation, Milford MA) coupled to a 5500 Triple Quad™ mass spectrometer (AB Sciex, Framingham MA). Chromatographic analysis was performed using a gradient elution of ammonium acetate with 0.1% formic acid and acetonitrile through an ACQUITY UPLC® HSS T3 column. The targeted analytes and internal standard were monitored using compound specific transitions based upon information collected in the TOF analysis. Quantitation was performed using analyte to internal standard response ratios compared to urine and serum calibrators fortified with U47700. The concentrations of the metabolites were estimated based upon the response of the parent drug relying on the assumption that the molecular response of the metabolites approximates the response of the parent drug.

Result: High resolution LC-MS analysis of the samples identified a peak in both samples at 329.1199 *m/z* corresponding to a monoisotopic molecular ion of $C_{16}H_{23}Cl_2N_2O^+$. The peak possessed an isotope pattern and retention time identical to a reference standard of U47700. Collision induced fragmentation generated a spectrum consistent with both the predicted theoretical fragmentation of U47700 and the fragmentation produced by the reference standard. Data mining of the high resolution chromatogram identified a series of demethylated and hydroxylated metabolites in the urine sample. Analysis of the high resolution fragmentation spectra allowed identification of the sites of demethylation. Four hydroxylated metabolites were identified occurring subsequent to each of the two demethylation reactions. Analysis of fragmentation patterns determined the location of the hydroxylation reactions to be localized on the cylclohexyl ring. No phase II metabolites were detected. U47700 was found to be the prominent circulating species (228 ng/mL) with trace amounts of desmethyl-U47700 (estimated concentration of 27 ng/mL). In urine, desmethyl-U47700 predominated (1900 ng/mL, estimated) followed by bisdesmethyl-U47700 (620 ng/mL, estimated), parent drug (390 ng/mL) and lower levels of 8 different hydroxylated metabolites (total estimated concentration of 690 ng/mL).

Conclusion/Discussion: Based upon this one overdose case involving U47700, we predict that U47700 is the optimal target in serum for detecting U47700. In contrast, desmethyl-U47700 and bisdesmethyl-U47700 appear to be the best urinary markers for detecting U47700 exposure.

Keywords: U47700, U4, Designer Opiates

S46

Quantification of Designer Drugs 5-F-ADB and Diphenidine in Postmortem Blood and Investigation of their Main Metabolic Pathways in Human by LC/Q-TOFMS and LC/MS/MS

Maiko Kusano*[1], Kei Zaitsu[1], Kentaro Taki[1], Saki Noda[1], Tomomi Ohara[1], Kazuaki Hisatsune[1,2], Tomomi Asano[1], Yumi Hayashi[3], Hitoshi Tsuchihashi[1], Akira Ishii[1]; [1]Department of Legal Medicine and Bioethics, Nagoya University Graduate School of Medicine, Nagoya, Japan, [2]Forensic Science Laboratory, Aichi Prefectural Police Headquarters, Aichi, Japan, [3]Department of Radiological and Medical Laboratory Sciences, Nagoya University Graduate School of Medicine, Nagoya, Japan

Background/Introduction: Despite the implementation of the new blanket scheduling system in 2013, designer drug abuse remains to be a serious social concern in Japan. Acute poisoning cases by overdosing on new designer drugs have been reported, compelling forensic toxicologists to develop rapid and sensitive detection methods for these drugs in biological specimens. Here we present an acute intoxication case involving methyl (S)-2-[1-(5-fluoropentyl)-1H-indazole-3-carboxamido]-3,3-dimethylbutanoate (5-F-ADB) and diphenidine. Targeted screening by liquid chromatography/tandem mass spectrometry (LC/MS/MS) allowed for the detection of very low level of unchanged 5-F-ADB in postmortem blood. Quantification and further metabolite investigation of 5-F-ADB and diphenidine was carried out to determine their main metabolic pathways in humans using LC/quadrupole time-of-flight MS (LC/Q-TOFMS).

Objective: To quantitate 5-F-ADB and diphenidine in postmortem blood, as well as to investigate their urinary metabolites using LC/Q-TOFMS and LC/MS/MS.

Method: 5-F-ADB and diphenidine standards were purchased from Cayman Chemicals (Ann Arbor, MI). One mL urine was enzymatically hydrolyzed overnight at 37°C with *H. pomatia* β-glucuronidase solution. A 300µL aliquot of the enzyme treated urine specimen was deproteinized with 900 µL of methanol, and centrifuged for 15 min at 16,000 g at 4°C. The supernatant was evaporated to dryness at 60°C under nitrogen. The residue was reconstituted in 60 µL of 70% methanol-10mM aqueous ammonium formate solution. For blood, 100µL of heart blood was deproteinized with 300µL methanol. The organic layer was evaporated to dryness under nitrogen and reconstituted with 25 µL of 30% methanol-10mM aqueous ammonium formate solution. Drug concentrations in blood were quantitated by standard addition. Analyses were performed either on a TripleTOF 5600 system or a QTRAP 6500 system (AB Sciex, Framingham, MA) coupled to a Shimadzu NexeraX2 LC (Shimadzu Co., Kyoto, Japan) system (L-column 2 ODS (1.5 x 50 mm, 3 µm) column; 10mM ammonium formate-5% (A) or 95% (B) methanol mobile phases; linear gradient; 0.15 mL/min flow).

Result: Initial screening by LC/Q-TOFMS in the information dependent acquisition (IDA) mode only detected diphenidine. Further urinary screening using our in-house database containing new designer drugs and metabolites detected 5-F-ADB and diphenidine metabolites; we thus performed a product ion scan (PIS) by LC/MS/MS in postmortem blood in search for the parent drug, and successfully detected 5-F-ADB. Quantification by standard addition (5-F-ADB: R^2=0.98 and diphenidine: 0.99 calibration curves) resulted in the blood concentrations to be 0.19 ng/mL for 5-F-ADB and 12 ng/mL for diphenidine. Investigation of the urinary metabolites revealed pathways involving ester hydrolysis (M1) followed by defluorination and hydroxylation on the pentyl chain (M2), amide hydrolysis followed by defluorination and hydroxylation on the pentyl chain (M3), as well as hydroxylation on the indole ring (M4). Mono- and di-hydroxylated diphenidine metabolites were also found.

Conclusion/Discussion: This is the first known report of 5-F-ADB quantification in postmortem blood and its human metabolism. Non-targeted screening by LC/Q-TOFMS and targeted screening by LC/MS/MS allowed for the detection of designer drugs present at very low blood concentrations. While targeted screening is imperative for detecting trace levels of drugs, in the present case, non-targeted screening of urinary metabolites led to the successful detection of unchanged 5-F-ADB in postmortem blood. Further, the present case demonstrates the importance of urinary metabolite screening for drugs with low blood concentration. Synthetic cannabinoids fluorinated at the terminal *N*-alkyl position are known to be extremely potent; while we suspect that the acute toxicity of 5-F-ADB is also high, it is unclear as to what extent the very low blood concentration of the drug contributed to the death of the deceased in the present case. Non-enzymatic and/or enzymatic degradation may play a role in decreasing the blood concentration of 5F-ADB, of which further investigation is currently underway.

Keywords: Designer Drug Metabolism, 5-F-ADB, Diphenidine

S47

Rapid Analysis of Ketamine and Xylazine in Rat Tissue by 2D LC/MS/MS Technology

Malorie Mella[2], Brendan Schweitzer[1], Sabra R. Botch-Jones[1], **Claude R. Mallet*[2]**; [1]Boston University School of Medicine, Boston, MA, [2]Waters Corporation, Milford, MA

Background/Introduction: In the field of veterinary medicine, xylazine is an approved compound by the Food and Drug Administration as an animal tranquillizer and often used in combination with ketamine. Since both drugs exhibit anesthetic properties, their recreational usage has also been reported in drug-facilitated sexual assaults cases. The increase in illicit usage prompted a re-classification of ketamine as a Schedule III drug in the United States Controlled Substance Act. Further, in post-mortem forensic toxicology casework, complex matrices can be difficult to analyze due to time-consuming extraction processes. Thus, in most tissue applications, liquid-liquid extraction (LLE) and solid phase extraction (SPE) are commonly used as de-fatting and cleanup/concentration steps, respectively. With complex matrices, a more robust extraction and clean up methodology is required to reach target limit of detection (LOD) and to maintain instrument performance. The analysis of xylazine and ketamine in biological tissue specimens (brain, heart, lung, liver, kidney, spleen, stomach contents) entails several analytical challenges, predominately during the extraction phase. As with all solids matrices, the sample must undergo a complete disruption of the cell membrane to expose the inner portion of tissue cells. Homogenization is the first step to extract a target analyte into a liquid solution prior to further sample clean up and concentration. Forensic laboratories often employ extensive and time consuming sample preparation protocols to reach sub parts per billion (ppb or ng/mL) levels. Advances in analytical capabilities with hyphenated instrumentation platforms have increased sensitivity to detect trace levels of analytes. Therefore, today's analytical challenge resides with the sample preparation techniques. Traditional solid phase extraction techniques often require a lengthy evaporation step, which will inevitably delay analysis. A micro extraction protocol combined with a multi-dimension chromatography can decrease sample preparation time without sacrificing the quality seen with current single dimension chromatography techniques.

Method: Biological tissue specimens, including brain, heart, lung, liver, kidney, spleen, and stomach contents, were taken from 10 rat specimens, which were dose with xylazine and ketamine before being euthanized. After homogenization, the extraction process was performed using a mixed mode reversed-phase/ion exchange sorbent. The mixed mode approach yields two eluting fractions, one fraction will comprise of neutral and acidic entities and the other fraction will concentrate the analytes with basic functionalities. When coupled to a 2D LC/MS/MS technology, several extraction steps can be eliminated from the protocol. Large volumes of organic extracts can be injected and pre-concentrated which allowed the elimination of the reconstitution and evaporation to dryness steps from the protocol. In this study, ketamine and xylazine were extracted from rat tissues using three extraction protocols for performance evaluation. The chosen 2D LC/MS/MS method used in this application was identified using a 6x6 automated methods development protocol, which target several key chromatography parameters for a total of 144 LC/MS/MS methods. The method evaluation was completed within 72 hours.

Result: The manual extraction of tissue samples were completed in less than 30 minutes. The analysis was performed using 100 uL of the final organic solvent (MeOH or ACN) extracts. The signal intensity for a 1 ppb is also very strong, suggesting a LOD in the low part-per-trillion range (ppt). The limit of quantification (LOQ) for all drugs measured at 100 ng/g (100 ppt) from a 1 g sample mass. The recovery values calculated from ion ratio (target analyte/deuterated internal standard) gave values of 98% and 93% for Ketamine and Xylazine in liver extract, respectively. The calibration curves (0.05 to 10 ng/g range) in liver extracts gave excellent linearity with R^2 values of 0.998 for both Xylazine and Ketamine. The chemistries used for this application gave an excellent performance well over 1000 injections.

Conclusion/Discussion: The micro extraction protocol of tissue samples in combination with a multi-dimension chromatography produced excellent results. The sample preparation was completed within 30 minutes using a 100 uL injection volume. Overall, the extraction and analytical method gave great CV's values (coefficient of variations) of less than 3.5 % for both target analytes.

Keywords: 2D LC/MS/MS, Bio-Analysis, Rapid Analysis

S48

Havoc™ in the Kitchen, Baking to get Baked, Preparation of Quality Control Materials

Carl E. Wolf*[1], Justin L. Poklis[2], Alphonse Poklis[1,2]; Departments of [1]Pathology and [2]Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA

Background/Introduction: The legalization of marijuana in the United States by state governments for both medicinal and recreational use has increased in the past few years. Currently 24 states have legalized marijuana for medicinal use. The US Drug Enforcement Administration (DEA) has classified marijuana as a Schedule 1 substance. Federally, the US Food and Drug Administration (FDA) does not regulate formulations of marijuana or marijuana constituents other than Marinol®, the only legal formulation of delta-9-tetrahydrocannabinol (THC). The FDA does not regulate formulations or packages of marijuana that are currently marketed in states with legalized marijuana. Marijuana edibles or "medibles", are a common form of packaging for medicinal and recreational marijuana use. Colorado classifies a serving of THC as 10 mg with a maximum of 100 mg per package. Oregon classifies a serving of THC as 5 mg with a maximum of 50 mg per package.

Objective: To evaluate the effects of brownie matrix and the baking process have on preparation of matrix matched QC materials used in the analysis of medibles and the stability of baked brownie QC materials.

Method: Baked QC materials were prepared according to the manufacture's recipe, Duncan Hines®. A box prepares 20 brownie servings or ~48 brownie bites. A 5 mg THC serving would equate to a 2 mg THC bite, and a 10mg THC serving would equate to a 4 mg THC bite. QC bites were prepared at 5 and 10 mg equivalent servings of THC& CBD. The cannabinoids were mixed in and portioned into bite cups, and baked at 300 ^{0}F. The remaining batter was baked for use as drug-free matrix. Sample preparation was a modified Washington State Police method. Ten ng of THC-d3 was added to a 25 mg sample and 2 mL of methanol. The sample was vortexed for 1min, stand for 2 min, centrifuged at 10,000g for 5min., and transferred to an autosampler. Instrumentation was a Waters Acquity UPLC with a TQD mass spectrometer (Waters Corporation, Milford, MA) with a Zorbax Eclipse XDB-C18, 3.5 um, 4.6 x 75 mm column. (Agilent Technologies, PA). The mobile phase was 20 mM ammonium formate in water (A) and 20 mM ammonium formate in methanol (B) isocratic 10:90 for 8min followed by a gradient to 0:100 over 1min. Samples were analyzed using a seven point calibration curve ranging from 0.8–80 µg/g THC and CBD. Validation QC materials (0.8, 2.4, 24 & 60 µg/g) were used to evaluate intra and inter-run bias. Samples were analyzed to evaluate carry-over, process efficiency, interferences and stability. Ten prepared and purchased brownies were used to evaluate selectivity, fortified at 5 µg/g (n=3).

Result: The assay was linear from 0.8–80 µg/g with r^2 >0.9996 for THC and CBD (n=3). Intra-run bias for QC materials ranged from -18% (0.8 µg/g THC) to 9 (0.8 µg/g CBD) (n=3). Inter-run bias for QC materials ranged from -10% (0.8 µg/g THC) to 5 % (24 µg/g THC) (n=9). No carry-over was detected. Process efficiency was 92–95 %. CBC, CBG, THCA, THCA-A and THCV did not interfere with the analysis. No interferences were detected from the 10 prepared and purchased brownies, fortified ranged from 4.9–5.8 µ/g (<0.7 SD). Samples were stable up to 72 hours after preparation. Baked QC 5 or 10 µg/g THC & CBD inter-run biases were 5.1 (SD=0.5) & 5.1 (SD=0.4), or 11.1 (SD=1.5) & 10.8 (SD+1.7), respectively.

Conclusion/Discussion: Brownie matrix and the baking process don't have an effect on the analysis of medibles. Baked brownie QC materials are stable for a minimum of 1 month.

Keywords: Medibles, Cannabinoids, QC Material

S49

Data-Mining the Poison Control Centers' Annual Reports (2000-2013) for Autopsy Blood Concentrations of the Primary Contributing Drug or Alcohol

David E. Moody[1*], Barbara I. Crouch[2,3], Olga Averin[1], Nena Bowman[2], Xiangyang Ye[3]; [1]Center for Human Toxicology, Department of Pharmacology and Toxicology, [2]Utah Poison Control Center, [3]Department of Pharmacotherapy, University of Utah, Salt Lake City, UT

Background/Introduction: The American Association of Poison Control Centers (AAPCC) issues an annual report compiling data reported to the National Poison Data System (NPDS). Table 21 within this report lists fatal non-pharmaceutical and pharmaceutical exposures.

Objective: We are data-mining the primary pharmaceutical or alcohol exposures in tables published from 2000 to 2013. Autopsy blood (whole, serum, plasma or unspecified) concentrations for the primary contributing drug were provided with ≈ 14% of cases. Mining this portion of the data-base allows us to tabulate blood concentrations along with corresponding demographics and listed co-ingestants; to assess the percent of blood cases reported in different drug categories, the number of suicide versus non-suicide cases within categories, and for the largest class, opioid analgesics, to assess the potential impact of multiple exposures to other opioids, ethanol and/or benzodiazepines.

Method: Cases with autopsy blood concentrations for the primary drug (or alcohol) were collected along with other demographics and additional substances. These were compiled into a single sortable table to examine numbers within categories and concentration means for selected comparisons.

Result: Fatal case listings grew gradually from 2000-2010 (1000-1100 per year); a surge of indirect reports (not calls to PCCs) for "analgesic" and "street and stimulant" (S&S) drug categories doubled listings for 2011-2013. Inclusion of autopsy blood concentrations was not consistent, varying from 5 to 15% of total cases, with higher inclusions at just over 20% in 2008 and 2010 and 40% in 2011. In total 2,474 blood cases were collected out of a total of 16,789 fatality cases. The percent distribution of blood cases amongst drug categories was consistent; the number of "total cases" and "blood cases" in the top categories were: analgesics (7165, 1298), S&S drugs (2383, 366), antidepressants (1693, 279), cardiovascular drugs (1632, 131), sedative/hypnotic/antipsychotic (S/H/A) (1145, 151) and alcohols (793, 103). Blood cases from suicides were very high from cardiovascular (91.6%), antidepressant (74.9%) and S/H/A (61.6%) drug categories, while low from analgesic (19.8%), alcohol (8.7%) and S&S (7.1%) categories. Within analgesics, blood cases and cases that were suicide were: opioids (79.9%, 14.1%), acetaminophen opioid combination (14.0%, 33.7%), acetaminophen ± non-opioid (3.2%, 64.3%) and NSAIDs (2.9%, 92.1%). Further investigation focused on the top five opioids ± acetaminophen with the following number of cases: methadone, 322; oxycodone ± acetaminophen, 295; hydrocodone ± acetaminophen, 169; morphine, 161; and fentanyl ± droperidol, 129. Morphine concentrations were listed as either "free" (80) or were undesignated (81); the latter were significantly higher and treated separately. In all opioid categories, concentrations for suicide cases were significantly higher and were excluded from further analyses. Potential pharmacodynamic drug interactions were tested for assuming the group mean concentration would be lower for an opioid and select co-medications. Opioids were coded for having another opioid, a benzodiazepine and/or ethanol listed as a co-medication. This initial comparison of cases without any of the selected co-medications versus those that contained any combination showed a trend for lower blood concentrations for all opioids that reached significance for oxycodone ($p=0.00015$) and hydrocodone ($p=0.0080$). More refined coding was made for: additional opioids (#2), additional benzodiazepines (#3), ethanol (#4), and opioids plus benzodiazepines (#5). Combinations #2 ($p=0.0034$), #3 ($p=0.0271$), #4 ($p=0.0424$) and #5 ($p=0.0031$) were all significant for oxycodone. Combinations #2 ($p=0.0073$) and #5 ($p=0.0425$) were significant for hydrocodone ($p=0.0073$). In contrast it was combination #4 that was significant for methadone ($p=0.0367$).

Conclusion/Discussion: Autopsy blood concentrations are commonly presented in forensic toxicology case studies. Data-mining the PCC annual reports from 2000-2013 compiled 2,474 such cases. This number was sufficient to allow examination of the potential pharmacodynamics drug interactions and substantiate the danger of combining opioids ± benzodiazepines.

Keywords: Data-Mining, Drug and Alcohol Fatality, Autopsy Blood Concentration

S50

Postmortem Tissue Distribution of Morphine, Morphine-3-glucuronide and Morphine-6-glucuronide in a Series of Heroin Deaths

Peter D. Maskell[1,2]*, Nathan E. Wilson[1], L. Nitin Seetohul[1,2], Giorgia De Paoli[1]; [1]Centre for Forensic and Legal Medicine, University of Dundee, Dundee, UK, [2]Department of Chemical and Forensic Sciences, University of Huddersfield, Huddersfield, UK, [3]School of Science and Technology, Nottingham Trent University, Nottingham, UK

Background/Introduction: Heroin is an opioid analgesic that is abused by around 16.5 million people worldwide and in 2011 was responsible for an estimated 211,000 deaths. The use and abuse of heroin is changing around the world. Europe is currently seeing a long term decline in the use of heroin. However increases in the abuse of heroin in other parts of the world such as North America and South West and Central Asia are being seen. This shows that heroin abuse continues to be a worldwide problem to be addressed. Once heroin is absorbed into the body it is rapidly metabolized to 6-acetylmorphine (6-AM). 6-AM is then metabolized to morphine with the final major steps of metabolism being the formation of the pharmacologically inactive morphine-3-glucuronide (M3G) and the pharmacologically active morphine-6-glucuronide (M6G). To date there is a lack of data on the levels of the glucuronide metabolites of morphine in heroin related deaths especially in postmortem tissue samples. In order to help with interpretation of heroin/morphine related deaths we determined the levels of morphine, morphine-3-glucuronide and morphine-6-glucuronide in a total of 44 postmortem cases between 2010 and 2012 in Scotland in which heroin intoxication was the cause of death (the presence of 6-AM in any of the postmortem samples collected was used as confirmation of heroin use).

Method: A validated LC/MS-MS method using solid phase extraction was used to quantitate morphine, M3G and M6G. The samples in which drugs were quantitated were femoral blood, cardiac blood, brain (thalamus), liver (deep right lobe), bone marrow (sternum) muscle (thoracic psoas) and vitreous. They were all collected as part of routine autopsy procedure. The limit of quantitation for all analytes was 0.01mg/L.

Result/Discussion: 79.5% of heroin deaths were male with 20.5% being female. The median age was 35 years old (range 21-65 years old). Table 1 shows the median (with range) of the concentrations of morphine, M3G and M6G detected. There were 4 cases in muscle (3 cases bone marrow) where there was no morphine, M3G or M6G detected, even though it was detected in other samples in the case.

Table 1:

		Fem Blood (mg/L)	Cardiac Blood (mg/L)	Liver (mg/kg)	Muscle (mg/kg)	Bone Marrow (mg/kg)	Brain (mg/kg)	Vitreous (mg/L)
Morphine	Median	**0.14**	**0.21**	**0.48**	**0.13**	**0.12**	**0.18**	**0.08**
	Mean	**0.20**	**0.30**	**0.65**	**0.21**	**0.26**	**0.26**	**0.11**
	Range	**0.01-1.35**	**0.01-1.85**	**0.01-2.55**	**0.01-1.13**	**0.01-3.23**	**0.01-1.96**	**0.01-0.49**
M3G	Median	**0.14**	**0.24**	**0.39**	**0.07**	**0.01**	**0.01**	**0.10**
	Mean	**0.23**	**0.38**	**0.71**	**0.09**	**0.06**	**0.07**	**0.16**
	Range	**0.01-1.12**	**0.01-1.74**	**0.01-4.68**	**0.01-0.91**	**0.01-0.32**	**0.01-1.12**	**0.01-0.65**
M6G	Median	**0.04**	**0.08**	**0.21**	**0.01**	**0.01**	**0.01**	**0.02**
	Mean	**0.06**	**0.10**	**0.33**	**0.03**	**0.03**	**0.02**	**0.03**
	Range	**0.01-0.66**	**0.01-0.48**	**0.01-1.76**	**0.01-0.15**	**0.01-0.1**	**0.01-0.06**	**0.01-0.19**

Conclusion: It may not be possible to detect heroin/morphine use in cases with limited samples (such as decomposed bodies). The information given here should allow some degree of interpretation of morphine results when postmortem samples may be lacking. The concentrations of morphine seen in postmortem cases overlap significantly with those seen in previously reported DUID cases. Postmortem cases should be interpreted with as much case knowledge as possible.

Keywords: Morphine, Heroin, Postmortem

S51

Estimation of Postmortem Interval Using Vitreous Potassium in Cases of Fatal Road Traffic Collisions

Suzanne Foster*, Guy Rutty, Mike Biggs, Frances Hollingbury, Stuart Hamilton, Paul Smith, Stephen Morley; University of Leicester, UK

Background/Introduction: Vitreous potassium levels as measured post-mortem and their utilization in the prediction of post-mortem interval has been discussed in the literature and several formulae have been proposed previously. It has been recommended that this be done using linear regression with potassium as the independent variable and post-mortem interval as the dependent variable. It has also been suggested that the circumstances in which death occurred may have an impact on the accuracy of this method and a higher level of precision can be achieved when this is accounted for.

Objective: The objective was to use post-mortem vitreous potassium as the independent variable and post-mortem interval as the dependant variable to see if it is possible to produce a formula that can predict post-mortem interval using cases of deaths due to trauma sustained in road traffic collisions. This would then be compared with the formulae produced by other authors.

Method: Vitreous humor samples were taken from 78 individuals whom had died as a result of road traffic collisions in Leicestershire between 2010 and 2015. Samples were obtained from both eyes using a 10ml syringe and 18 gauge needles and decanted into a plain specimen container for post-mortem analysis. The specimens were subsequently sent for on-site analysis of electrolytes including potassium. Measurement of potassium was by an indirect ion specific electrode Siemens diagnostics ADVIA 1800 Chemistry System. The exact time of death was noted from police reports, the time of post-mortem was recorded and a post-mortem interval was calculated to the nearest two decimal points. Samples from individuals where a precise time of death was not established were not included in the study. Linear regression was then used to analyse the relationship between the two.

Result: For the 78 cases post-mortem interval fell between 6 and 162 hours. The mean post-mortem interval was 74.2 hours with a standard deviation of 39.4. It was found that as vitreous potassium increases the post-mortem interval also increases; exhibiting a linear relationship between the two. Correlation between the two is illustrated by a regression equation obtained from our data of $PMI = 6.42[K+] - 40.94$ with an R^2 of 0.67 ($p < 0.001$). This has produced a formula which is comparable with those proposed in previous literature.

Conclusion/Discussion: Validated methods have been used to produce a formula for prediction of post-mortem interval using vitreous potassium. Although this is specific to road traffic collisions methods are transferable and can be seen to be comparable with other recently published methods. Nonetheless, even with the use of these equations the level of accuracy may still not meet expectations and it is suggested that biomarkers delivering a higher level of precision should still be sought.

Keywords: Postmortem Interval, Road Traffic Collision, Vitreous Potassium

S52
Postmortem Findings in a Series of Acetyl Fentanyl Deaths

Robert Kronstrand*, Markus Roman, Emma Rapp, Ariane Wohlfarth; National Board of Forensic Medicine, Department of Forensic Genetics and Forensic Toxicology, Linköping, Sweden

Background/Introduction: Acetyl fentanyl has been available for a few years worldwide but not encountered in Sweden until April 2015 when the first occurrence of a non-fatal intoxication was reported. Acetyl fentanyl is one among several fentanyl analogs that are sold over the internet. It is less potent than fentanyl but still 15 times more potent than morphine and has been assigned to several deaths in Asia, Europe and USA. In Sweden acetyl fentanyl was scheduled as a narcotic drug on August 18th 2015. Even though it had a relatively short time as a legal drug in Sweden there were several intoxications reported to the Swedish poison center, and it has also been detected in several autopsy cases.

Objective: The objective of this study was to describe the post mortem findings of deaths involving acetyl fentanyl and to find possible risk factors and significant findings that may help in interpretation.

Method: All cases positive for acetyl fentanyl during 2015 were included in the study (total case load 5511). Routinely, autopsy cases are subject to a comprehensive UHPLC-TOF screening in femoral blood for medications and drugs of abuse including acetyl fentanyl. All positive findings are confirmed. Acetyl fentanyl was quantified using LC-MS/MS. Briefly, 0.5 g of blood was fortified with d5-fentanyl as internal standard and the sample was precipitated with 0.75 mL of acetonitrile/ethanol (90:10) containing 0.075% formic acid. Data acquisition was performed in positive ionization mode and two transitions were measured. Transition ratios and retention time was used for identification. The LOQ was 0.001 µg/g. The repeatability was 3% at low level and 5% at high level with accuracy of 100% and 96%, respectively.

Result: Thirty-two cases were found positive for acetyl fentanyl with an overrepresentation of males (N=27). Only two cases had acetyl fentanyl as the only finding. Most cases had several other positive findings of medications as well as drugs of abuse. Eleven were also positive for ethanol. All cases but one had fatal intoxication with acetyl fentanyl ascribed as the cause of death (N=26) or as contributing to death (N=5). The femoral blood concentrations ranged from 0.007 to 0.77 µg/g (median 0.16 µg/g) in cases attributed to acetyl fentanyl and from 0.004 to 0.34 µg/g (median 0.13 µg/g) in cases where acetyl fentanyl contributed to death. The most common post mortem finding was pulmonary congestion/edema reported in 22 cases (lung weight median 1453 g and mean 1445 g). Froth in the airways, a common finding in opiate overdose deaths, was seldom seen. Circumstances were dominated by the subject being found dead and only 5 were witnessed deaths where paramedical treatment or intensive care was unsuccessful. The acetyl fentanyl formulations were powders, tablets as well as nasal spray containers. The findings suggested respiratory depression as the main mechanism of toxicity.

In the living, acetyl fentanyl concentrations from 0.0006 to 0.052 µg/mL serum have been reported (N=7) with effects including respiratory depression, unconsciousness, tachycardia and aspiration. There was an overlap between the concentrations in our cases, but the majority (N=25) had concentrations higher or much higher than the maximum concentration reported in the living. Our cases also present with concentrations similar to other reported fatal intoxications.

Conclusion/Discussion: We conclude that acetyl fentanyl is a high risk drug of abuse and unless a person quickly receives intensive supportive care, the outcome is unfavorable. This study shows that the use of acetyl fentanyl alone is a risk factor, which is often increased with concomitant consumption of other drugs. There were few significant and specific post mortem findings and the concentrations were found to overlap with surviving cases.

Keywords: Acetyl Fentanyl, Postmortem, Fatal Intoxication

S53
The Role of Methadone In Two Separate Child Fatality Cases

Craig Chatterton*; Office of The Chief Medical Examiner, Edmonton, AB, Canada

Background/Introduction: Methadone is a potent pain-killing drug used for the alleviation of very severe pain though it is more commonly used as a substitute drug in the treatment of opiate addiction. Two separate case studies are presented which detail the ingestion/administration of methadone to young children. A fatal outcome occurred in each case. The objective for the case study presentation is to highlight the added values of drugs in hair analysis.

Objective: The objective of this study was to audit the number and nature of occurrences in post-mortem situations.

Method/Results: Case 1 (Bristol, UK): A 14-month old child was found, in a cot, unresponsive and not breathing. The child was declared dead on arrival at hospital. The police investigation which followed revealed that both the child's parents were prescribed methadone and that the drug was stored (and administered) using a syringe/plunger apparatus. Post mortem samples, including blood, hair and urine were collected from the child; hair and urine was collected from the child's older sibling. Toxicology samples were submitted to the Forensic Science Service in Lancashire, England. Toxicology analysis identified methadone (0.28mg/L) and EDDP in the post mortem blood (GC-MS) and hair (LC-MS/MS). Acetaminophen was detected (but not quantified) in the sibling's urine; methadone and its metabolite were present in the sibling's hair (see tables below).

Drug	Segment	Result (ng/mg)
Methadone	0 to 3 cm	0.65
Methadone	3 to 6 cm	0.99
EDDP	0 to 3cm	0.03
EDDP	3 to 6 cm	0.02

Plucked head hair from the deceased

Drug	Segment	Result (ng/mg)
Methadone	0 to 7 cm	0.35
Methadone	7 to 18 cm	0.59
EDDP	0 to 7cm	0.02
EDDP	7 to 18 cm	0.01

Cut head hair from the sibling (1 month post incident)

Drug	Segment	Result (ng/mg)
Methadone	0 to 1 cm	0.04
Methadone	1 to 2 cm	0.26
Methadone	2 to 3cm	0.40
Methadone	3 to 4 cm	0.08
Methadone	4 to 5cm	0.04
Methadone	5 to 6 cm	0.04

Cut head hair from the sibling (3 months post incident)

Drug	Segment	Result (ng/mg)
EDDP	0 to 1 cm	N/D
EDDP	1 to 2 cm	0.03
EDDP	2 to 3cm	0.03
EDDP	3 to 4 cm	<LOQ
EDDP	4 to 5cm	<LOQ
EDDP	5 to 6 cm	<LOQ

Cut head hair from the sibling (3 months post incident)

The death of the child was attributed to complications from a rare and lethal form of chicken pox/bronchial pneumonia. Methadone was ruled out as a contributing factor, however the parents did admit to 'rubbing' methadone into the child's gums during the fall, to soothe the child who was teething at that time. The mother was jailed for 15 months; the father was jailed for 18 months.

Case 2 (Alberta, Canada): A 21-week old infant, who had recently been suffering with a 'cold', was breast fed by its mother at approximately midnight and was subsequently put to bed in the master bedroom. At approximately 09:00hrs the following morning, the cold, stiff body of the infant was discovered lying next to its mother in bed. Emergency services attended and confirmed the infant to be deceased at the scene. At the time of the child's death, the mother was prescribed methadone (260 mg daily) on a monitored program.
Toxicology analysis of post mortem samples that were collected from the infant was undertaken at the Office of the Chief Medical Examiner in Edmonton, AB, Canada. The samples were analyzed using various analytical techniques including: enzyme linked immunosorbent assay (ELISA), gas chromatography mass spectroscopy (GC-MS), liquid chromatography tandem mass spectroscopy (LC-MS/MS) and liquid chromatography time of flight (LC-TOF). Analysis identified and quantified methadone in the infant's post mortem blood (cardiac: 0.12mg/L – EDDP not detected), vitreous (approx. 0.05mg/L), liver (right lobe: 0.62 mg/kg) and gastric contents (0.01mg/23g). No other drugs were detected.

There is no convincing case reported in the scientific literature of an infant death from methadone poisoning through the ingestion of breast milk. The cause of death was 'undetermined'.

Conclusion/Discussion: These two case studies highlight the difficulties which face the post-mortem forensic toxicologist, when trying to offer a detailed interpretation of analytical toxicology results.

Keywords: Postmortem, Drugs in Hair & Breast-Milk, Methadone

S54

A Potential Application of Inferential Statistics to Assist in the Interpretation of Postmortem Drug Concentrations

Dwain C. Fuller*[1], Sandra C. Bishop-Freeman[2], Ruth E. Winecker[2]; [1]Forensic Toxicology Consultant, Mansfield, TX, [2]NC Office of the Chief Medical Examiner, UNC-SOM-Dept. of Pathology and Laboratory Medicine, Raleigh, NC

Background/Introduction: The interpretation of postmortem drug concentrations can be difficult, and statistical treatments of such data in the literature have been few. The 2009 National Academy of Sciences provided impetus to make forensic science "more scientific".

Objective: In response, the authors propose a potential application of inferential statistics, which could be used to mathematically compare incidental postmortem drug concentrations, serving as surrogates for therapeutic concentrations, with lethal postmortem drug concentrations to produce a "prior probability" that a given postmortem drug finding constituted a lethal event. The authors refer to this as a "causality index."

Conclusion/Discussion: The causality index is not intended to stand alone, but should be considered an additional tool to be applied within the context of other findings such as pathology, history, potential tolerance, and other variables. The success of such a paradigm requires the collection of well-controlled, statistically-representative data relating to drug concentrations. The authors will present some real-life examples of causality indices.

Keywords: Postmortem, Interpretation, Statistics

P01
Evaluation of the 20-Target DUID Biochip with the Randox Evidence Analyzer

Curt E. Harper*, Rebekah Boswell, Kristen Ellis, Jade Grimes; Alabama Dept. of Forensic Sciences, Pelham, AL

Background/Introduction: The Randox Evidence Analyzer offers a solution for drug screening in drug-impaired driving and motor vehicle fatalities with its biochip array technology for competitive immunoassay testing. The Analyzer is a fully automated instrument with the ability to streamline drug screening. We previously validated a 17-target custom (CST XVI) biochip. In January 2016, Randox released their latest 20-target DUID Biochip. Our original custom biochip served as the foundation for the new DUID Biochip. There were three major changes to our original custom biochip: addition of PCP, the expansion of opioid detectability with Oxycodone 1 and 2, and the improvement of the methamphetamine antibody. We set forth to evaluate these changes prior to implementation into casework.

Objective: To evaluate the new Randox Evidence Analyzer DUID Biochip in whole blood for DUI/D and postmortem testing and review its performance over six months. The 20-target custom biochip included the following assays: amphetamine, barbiturates, benzodiazepines 1 and 2, buprenorphine, cannabinoids, carisoprodol, cocaine, dextromethorphan, fentanyl, methadone, methamphetamine, opiate, opioids, oxycodone 1 and 2, PCP, tramadol, tricyclic antidepressants, zolpidem.

Method: For sample preparation, 200 µL of whole blood was combined with 600 µL of buffer (4-fold dilution). The Analyzer contains an array of discrete test regions (DTRs) with immobilized antibodies specific to different drug classes. Competitive binding occurs between the enzyme-labeled conjugate and specimen. Luminol and peroxide function as the substrate creating a chemiluminescent light signal from each DTR. The light intensity is detected by digital imaging technology. Intra- and between-day precision was assessed in triplicate over five different runs at three different concentration pools: 50% below the decision point (low), at the decision point (cutoff), and 50% above the decision point (high) for PCP. We compared methamphetamine false positive rates between our custom biochip and the new DUID biochip in over 100 methamphetamine positive cases. We characterized the use of four antibodies (opiate, opioids, oxycodone 1 & 2) to identify opioids. We also reviewed over 2000 cases from 2016 to further document false positive/negative rates, instrument issues, and control trends. Lastly, we compared over 50 cases simultaneously screened by the Evidence Analyzer using our previous 17-target custom Biochip versus the new 20-target DUID Biochip to assess overall performance. As a component of their in-house validation, Randox evaluated the following parameters for all targets: precision, interference, limit of detection, and cross-reactivity.

Result: Precision (CV%) was less than 10% at the cutoff for PCP with no false positive or negatives identified. The use of four opioid antibodies enhanced the detectability and predictability of specific opioids, especially oxymorphone. This addressed a known limitation in our previous biochip. Methamphetamine false positive rates decreased 93% with the improved antibody. Methamphetamine false positive were 2% and 28% for the new and previous antibody, respectively. This was achieved while lowering the cutoff from 60 ng/mL to 20 mg/mL. Overall low false negative and positive rates for all targets indicate that the assay has sufficient precision around the cutoff to correctly identify a sample as positive or negative.

Conclusion/Discussion: The new DUID Biochip has proven to be a useful tool for drug screening in a high throughput laboratory. This method screens for all Tier 1 drugs listed in the "Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities" published in JAT. Laboratories should strive to enhance their scope of analysis for DUID testing. The shift from our previous biochip to the DUID biochip improved screening performance.

Keywords: Immunoassay, Randox, DUID

P02

Use of an Internal Hydrolysis Indicator for Monitoring β-Glucuronidase Activity

Lacy L. Taylor[1*], Brandy M. Hill[2], Noah A. Flint[2], Vinh Ma[2], Chantry J. Clark[2], Frederick G. Strathmann[1,3]; [1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]ARUP Laboratories, Salt Lake City, UT, [3]University of Utah Department of Pathology, Salt Lake City, UT

Background/Introduction: Consumed benzodiazepines (benzos) will undergo a process called glucuronidation, in which a UDP-glucuronosyl transferase enzyme utilizes UDP to attach a sugar moiety (glucuronic acid) to a benzo, forming a glycosidic bond [1]. Glucuronidation occurs in over 75% of metabolized benzos [2] in order to solubilize the drugs for urinary excretion. Often in clinical testing an enzymatic hydrolysis step is implemented to increase the sensitivity of benzos by hydrolyzing β-D-glucuronic acid from benzo-glucuronide conjugates in urine samples using the β-Glucuronidase enzyme. Hydrolysis alters benzos making parent drug origins difficult to determine. Resorufin β-D-glucuronide, a substrate of the β-Glucuronidase enzyme, was added to patient samples to determine if proper hydrolysis had occurred. The presence of resorufin as an internal hydrolysis indicator shows the activity and efficiency of the enzyme in each patient sample.

Objective: To address the clinical needs for drug monitoring and to provide a more sensitive and thorough result analysis using resorufin as an internal hydrolysis indicator.

Methods: Samples of synthetic urine or patient's urine were obtained and mixed with rapid hydrolysis buffer (Sigma-Aldrich, St. Louis, MO) containing 7 µg/mL resorufin β-D-glucuronide (Sigma-Aldrich). The β-Glucuronidase enzyme was added (~1600 units), and the reaction mixture was left to sit at room temperature (RT) for 15 minutes. After incubation, the enzyme was inhibited with sodium bicarbonate buffer (0.1 M, pH 9.0). The reaction mixture was then transferred to an SLE+ fixed well plate column (Biotage, Charlotte, NC), which was attached to a 96 deep well plate. The SLE+ fixed well plate column and attached 96 deep well plate was then transferred to an Automated Liquid Dispenser (ALD) instrument that loaded the sample onto the column (using positive gas pressure) and eluted the analytes with ethyl acetate. Samples were dried and reconstituted in 200 µLs of reconstitution buffer (9:1, 5 mM Ammonium Formate/Methanol Optima) in preparation for liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS) data analysis. Variables such as enzyme concentration, incubation time, and buffer conditions were tested.

Results/Discussion: Of the 668 patient samples tested, 284 samples showed a benzodiazepine to be present using an enzymatic hydrolysis step not previously observed when tested without a hydrolysis step (a positivity rate increase of 42.5%). The inclusion of resorufin as an internal hydrolysis control has proven useful in identifying enzymatic activity for each patient sample prepared. The appearance of resorufin by LC-TOF-MS in each sample indicates that hydrolysis has occurred, and therefore compounds that are glucuronidated have also undergone hydrolysis. β-Glucuronidase enzyme activity appears to be quite variable from significant differences observed in resorufin area counts between patient samples, even when run on the same day and batch. Recovery studies were done and loss was observed in samples predominantly by extraction using the SLE+ column (~79% average recovery). Although recovery issues may explain some of the resorufin fluctuation, there were a few examples were resorufin area counts were low but the analyte IS area counts were normal, suggesting other contributing factors. The cutoff for the TOF assay ranged from 2 ng/mL to 400 ng/mL, depending on the analyte of interest. Average imprecision (%CV) was within ± 15%. Validation results correlated well with the original method, suggesting that matrix effects, imprecision, recovery, and detection thresholds were not compromised by the added hydrolysis step.

Conclusions: Hydrolysis reactions greatly improve the sensitivity of benzodiazepines by LC-TOF-MS analysis. The use of an internal hydrolysis control (resorufin) is crucial for monitoring enzyme activity from patient-to-patient.

References:

1. Pavel Anzenbacher, a.U.M.Z., *Metabolism of Drugs and Other Xenobiotics*. 2012, Wiley-VCH Verlag & Co.: Weinheim, Germany.
2. Cravey, R.C.B.a.R.H., *Disposition of Toxic Drugs and Chemicals in Man.* Chemical Toxicology Institute, 1995. **4th edition**: p. 432-433, 569-570.

Keywords: Benzodiazepines, Glucuronidation, β-Glucuronidase

P03

Hydrolyzing 30,000 ng/mL of Codeine-6-β-glucuronide in 15 Minutes with Greater than 80% Efficiency using IMCSzyme®

Cathleen Melendez*, Margarita Marinova, L. Andrew Lee, Pongkwan Sitasuwan; Integrated Micro-Chromatography Systems, LLC, Columbia, SC

Background/Introduction: Enzymatic hydrolysis of glucuronides is common practice in many of the urine drug testing laboratories utilizing LC-MS/MS. Limitations on directly monitoring glucuronides on tandem mass spectrometry are higher cost of glucuronide standards and lack of certified reference materials of the glucuronidated drug. However, a major historical challenge for enzymatic hydrolysis has been in opiates. In particular, hydrolysis of codeine-6-β-D-glucuronide (CODG) is challenging and generally has poor yield even with incubation time over 2 hours. Although the cutoff concentration of codeine in urine is 2,000 ng/mL according to the guideline from the US Substance Abuse and Mental Health Services Administration (SAMHSA), the various combinations of drugs and their glucuronidated metabolites detected in actual patient urine samples could be as high as 100,000 ng/mL. This study examines the genetically modified β-glucuronidase, IMCSzyme®, and its ability to achieve over 80% recovery of glucuronides in as little as 15 minutes for over 30,000 ng/mL of various glucuronidated opiates in urine.
Objective: To determine the hydrolysis time and amount of IMCSzyme® needed to hydrolyze over 30,000 ng/mL of glucuronidated opiates with >80% recovery.

Method: Drug-free urine was spiked with 30,000 ng/mL of CODG and 500 ng/mL of morphine-6-β-D-glucuronide, oxymorphone-3-β-D-glucuronide, and hydromorphone-3-β-D-glucuronide. 30 µL of spiked urine was hydrolyzed with 270 µL of hydrolysis solution (containing rapid hydrolysis buffer, IMCSzyme®, water, and internal standards). The enzyme amounts in each hydrolysis reaction were varied from 5 µL to 80 µL. The incubation temperature was fixed at 55˚C for 15, 30, or 60 minutes. The hydrolyzed samples were extracted with DPX WAX tips and eluted with 1% formic acid in acetonitrile. The eluent was dried under nitrogen and reconstituted with 100 µL of 5% methanol in water before being analyzed on LC-MS/MS.

Result: The results illustrated that at least 60 µL of the enzyme was needed to achieve greater than 80% hydrolysis of 30,000 ng/mL CODG within 15 minutes. For 30-minute incubation, greater than 90% hydrolysis was achieved with 30 µL of IMCSzyme®. The minimum enzyme amount to hydrolyze greater than 80% and 90% of CODG within 60 minutes was 15 µL and 25 µL, respectively.

Conclusion/Discussion: Greater than 80% hydrolysis of 30,000 ng/mL of CODG and three other glucuronidated opiates at 500 ng/mL was achieved using different amounts of IMCSzyme® depending on the desired incubation times (15 µL for 60 minutes, 30 µL for 30 minutes, or 60 µL for 15 minutes). This reduction in hydrolysis time from 60 and 30 minutes to 15 minutes for such a large quantity of glucuronides is essential for increasing throughput while maintaining accuracy. The hydrolysis process is easily adapted for full automation.

Keywords: Codeine, IMCSzyme®, Urine Hydrolysis

P04

The Development of a Liquid Chromatography-Tandem Mass Spectrometry Method to Analyze Hydrocodone and its Metabolites

David M. Andrenyak*, Wenfang B. Fang, David E. Moody; Center for Human Toxicology, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT

Background/Introduction: Over the past two decades, therapeutic and illicit use of opioid analgesic drugs has increased. Hydrocodone (HYCOD) is one of the more popular opioid analgesics. HYCOD is usually taken orally in formulations where it is combined with non-opioid analgesics such as acetaminophen; however a single-entity extended release formulation has recently entered the market. HYCOD is metabolized by O-demethylation to hydromorphone (HYMOR), N-demethylation to norhydrocodone (NORHYC), and reduction of the keto group to dihydrocodeine (DHCOD). Long-term use of HYCOD can lead to dependence / addiction along with toxicity and death from overdosage. Widespread use of HYCOD presents the need for analytical procedures to analyze HYCOD and its metabolites in biological samples.

Objective: Develop a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the analysis of HYCOD HYMOR, NORHYC, and DHCOD in plasma samples.

Method: We initially attempted to employ a liquid-liquid extraction procedure that was successful for plasma oxycodone testing (Fang et al., JAT, 37:337-44), however the liquid-liquid procedure resulted in a low extraction recovery for HYMOR. Instead we applied a solid phase extraction (SPE) procedure that used Clean Screen® SPE columns (United Chemical Technologies, Bristol, PA) and elution with dichloromethane: isopropanol: ammonia (80:20:2) for processing the samples. Calibration range was 0.2 to 250 ng/mL for all the compounds. A 1 mL volume of each calibrator, control, blank, or sample was added to separate 16 X 100 mm glass culture tubes. Each tube was fortified with 5 ng of internal standards (50 µL of 0.1 ng/µL HYCOD-d_6, HYMOR-d_6, NORHYC-d_3, and DHCOD-d_6). Controls were prepared at 0.6, 10, and 200 ng/mL for each compound. The LC-MS/MS analysis used an Agilent (Santa Clara, CA) 1100 LC interfaced with an Access TSQ Quantum triple quad tandem MS and Xcalibur® version 2.0 SR2 software (Thermo Scientific, San Jose, CA). The LC column used was an YMC Pack ODS-AQ, 100 X 2 mm (YMC AMERICA, Inc., Allentown, PA). The isocratic mobile phase was 0.1 % formic acid: methanol (85:15) at 0.12 mL/minute flow rate. The MS/MS analysis used positive ion Electrospray and selected reaction monitoring (SRM). All of the precursor ions were the protonated molecular ions. The SRM transitions used for the quantitation were HYCOD: 300→199; HYCOD-d_6: 306→202; HYMOR: 286→185; HYMOR-d_6: 292→185; NORHYC: 286→199; NORHYC-d_3: 289→202; DHCOD: 302→199; DHCOD-d_6: 308→202.

Result: Good chromatographic peak shape was achieved for each compound. Evaluating 100 ng/mL standards, the initial attempt to use liquid-liquid extraction showed an extraction recovery of greater than 50% for HYCOD, NORHYC, and DHCOD but less than 10 % for HYMOR. Processing with SPE, HYMOR recovery improved to 56 %, SPE recovery for the other compounds were – HYCOD: 86 %; NORHYC: 62 %; and DHCOD: 86 %. Ion suppression evaluation showed that the blank plasma SPE extracts reduced the MS/MS signal as -– HYCOD: -9 %; HYMOR: -34 %NORHYC: -13 %; and DHCOD: -14 %. Since deuterated isotopologues of the compounds were used as internal standards, any ion suppression would have minimal impact on the accuracy of the analysis. Accuracy and precision were evaluated by analyzing the control samples. The intra-run precision ranged from 90.5 to 98.3 % of the target and the intra-run precision ranged from 1.8 to 8.8 %.

Conclusion/Discussion: An effective method for the analysis of HYCOD and its metabolites was developed. We plan to characterize the stability of the analysis and hope to apply the method to pharmacokinetic studies of HYCOD.

Funding: Supported by NIDA Contract N01DA-14-7788.

Keywords: Hydrocodone, LC-MS/MS, Plasma

P05

Validation of a Quantitative LC-MS-MS Method for Antiepileptic and Acidic Drugs in Postmortem Matrices

Rebecca L. Wagner*; Virginia Department of Forensic Science, Richmond, VA

Introduction: The Virginia Department of Forensic Science analyzes biological specimens for the presence or absence of drugs and alcohol in DUI/DUID, postmortem and non-implied consent cases including search warrant and drug facilitated sexual assault cases. The yearly statewide submission is approximately 8300 cases with 3000 DUI/DUID, 5000 postmortem, and 300 non-implied consent case submission profile. With increasing case submissions and limited sample volume, it is imperative to develop and validate methods that are efficient, comprehensive, and require small sample volumes. A quantitative method for the analysis of antiepileptic drugs supports compliance determinations for medicolegal death investigations. A quantitative liquid chromatography tandem mass spectrometry (LC-MS-MS) method has been validated for nine antiepileptic and acidic drugs in whole blood and postmortem samples. The validated method enables a more comprehensive and efficient analysis scheme compared to the previously required multi-method analysis scheme.

Objectives: To develop and validate a quantitative LC-MS-MS method for the analysis of nine antiepileptic and acidic drugs including: gabapentin, levetiracetam, lamotrigine, zonisamide, 10,11-dihydro-10-hydroxycarbazepine (oxcarbazepine metabolite), oxcarbazepine, topiramate, carbamazepine, and phenytoin in whole blood and other available postmortem matrices.

Methods: A thorough validation was conducted for each target compound within the method. The validation was completed in accordance with the Scientific Working Group for Forensic Toxicology (SWGTOX) method validation guidelines and Virginia Department of Forensic Science validation requirements. Aspects evaluated within the validation were accuracy and precision, sensitivity, linearity and calibration model, suppression/enhancement, recovery, carryover, interferences, dilution integrity, and post-extraction stability.

Results: A methanolic protein precipitation extraction was validated using 200 µL of sample and 1.0 mL of methanol. Samples were vortexed for approximately 15-30 seconds and centrifuged for 15 minutes at approximately 2800 rpm prior to positive mode dynamic MRM LC-MS-MS analysis. All target compounds passed the comprehensive validation. Accuracy and precision for all compounds was within the predetermined acceptance criteria of pooled accuracy and precision within ±20 % at three concentration levels within the calibration range. The best fit calibration model was established for each compound using statistical analysis, residual plots, and coefficient of determination. The best fit calibration model for all compounds with the exception of levetiracetam and phenytoin was a weighted (1/x) quadratic model. Levetiracetam and phenytoin calibration models were best fit with weighted (1/x) linear calibration models. The calibration range for all compounds was 1.0-40.0 mg/L with a limit of detection of 0.125 mg/L. The limit of quantitation was 0.125 mg/L for gabapentin, levetiracetam, and lamotrigine. Zonisamide, oxcarbazepine metabolite, oxcarbazepine, topiramate, and carbamazepine all had a 0.250 mg/L limit of quantitation while phenytoin had the highest limit of quantitation (1.0 mg/L). In whole blood samples, the ion suppression raged from 32-48% suppression. In liver and urine samples, the suppression ranged from 14-48% and 13-48%, respectively. The methanolic protein precipitation produced a recovery of approximately 50% for all analytes and respective internal standards. Although suppression and a decrease recovery were noted during the validation the factors did not impact the overall accuracy and precision of the method.

Conclusions: The method development and validation of a quantitative method for the analysis nine antiepileptic and acidic compounds using LC-MS-MS was successful. The comprehensive method is a sensitive technique that requires less sample volume and is more efficient than previous methods employed by the Virginia Department of Forensic Science. The rapid methanolic protein precipitation extraction in conjunction with a 13 minute analysis time enables increased laboratory efficiency. Therefore, the fully validated method is another technique that can aide in streamlining forensic toxicology analysis.

Keywords: Quantitative Method Validation, Tandem Mass Spectrometry, Antiepileptic Drugs

P06
Quantitation of Ethanol in Whole Blood by HS/GC-MS

Michael Weaver*, Curt Harper, Dina Swanson; Alabama Department of Forensic Sciences

Background/Introduction: The identification and quantitation of ethanol and related volatiles by headspace gas chromatography-flame ionization detector (HS/GC-FID) has proven to be a robust methodology over the last several decades. Although ethanol is the most commonly detected drug in forensic toxicology, it remains one of the few analytes not routinely confirmed by mass spectrometry. Few methods have been developed using headspace gas chromatography-mass spectrometry (HS/GC-MS) to identify and/or quantitate ethanol and other volatiles. The identification by mass spectrometry adds an additional layer of defensibility in court.

Objective: To validate a method to quantitate ethanol and related volatiles (acetone, isopropanol, and methanol) by HS/GC-MS.

Method: We utilized an Agilent 7890A Gas Chromatograph with a 5975C Mass Spectrometer Detector (triple axis detection) and a G1888 70-capacity autosampler. 100 µL of sample was mixed with 1 mL of n-propanol and placed in a 20 mL headspace vial, sealed, and placed on the instrument for analysis. Calibrators and controls were purchased from Cerilliant, Lipomed and NIST. Accuracy and precision were assessed in quadruplicate over at least five different days at 0.01, 0.025, 0.05, 0.08, 0.15, and 0.30% for ethanol and 0.05% for other volatiles. Individual controls were deemed acceptable if they agreed with their respective target within the greater of 0.005% or 5%. Previously analyzed samples were evaluated for overall method accuracy by comparison with HS/GC-FID results. Results were expressed as the ratio (R) of the test concentration (C_{test}) to the target concentration (C_{target}). Carryover, interference, in-vial dilution, and robustness were also assessed.

Result: Linearity was established from 0.01% - 0.50% for ethanol using 1/x weighting. LOD and LLOQ were 0.01%. LOD was administratively set and not evaluated below 0.01%. Accuracy ranged from (R=0.945-1.036) for ethanol (see table below). Precision (CV %) was less than 5% at all concentrations for ethanol (see table below).

Ethanol	0.010%	0.025%	0.050%	0.080%	0.150%	0.300%
n	20	20	34	26	26	26
Accuracy (R)	1.013	0.953	0.945	0.990	0.997	1.036
Precision (%CV)	2.62%	3.25%	2.25%	4.37%	2.01%	3.84%
Acceptable Range	0.005-0.015	0.020-0.030	0.045-0.055	0.075-0.085	0.143-0.158	0.285-0.315
% Acceptable	100%	100%	100%	88%	100%	81%

Overall accuracy and precision for ethanol in previously analyzed blood, urine, and vitreous specimens were R=1.006 and CV% =3.0%, respectively. All 97 analyses fell within +/- 10% of the target concentration with 88% within +/- 5%. Accuracy ranged from (R = 0.939-1.007) for related volatiles at 0.05%. Methanol, acetone, and isopropanol measured had CVs of 3.97%, 4.27%, and 2.40%, respectively. Expanded uncertainty for ethanol was calculated using precision data from a 0.08% whole blood control from UTAK.with a coverage probability of 99.7% (k=3) was 4.47%. No carryover was observed with up to 5% ethanol or related volatiles. No interferents were noted from blank matrices or commonly encountered volatiles. In-vial dilution at 2x and 3x produced accuracy and precision comparable to other previously analyzed specimens.

Conclusion/Discussion: In 2011, Tiscione et al. published a procedure for the quantitation of ethanol by FID with simultaneous qualitative confirmation by MS. We present a quantitation method for ethanol and related volatiles by HS/GC-MS with excellent precision, accuracy, sensitivity, and specificity. This method could be implemented into a number of workflows for routine screening and confirmation to further enhance the confidence in alcohol identification.

Keywords: Ethanol, HS/GC-MS, Method Validation

P07

Quantitative Analysis of THC and Related Cannabinoids in Multiple Matrices Using a Novel Solid Phase Extraction Sorbent Coupled with UPLC/MS/MS for Clinical and Toxicology Applications

Xin Zhang*, Jonathan Danaceau, Erin Chambers, Kim Haynes; Waters Corporation, Milford, MA

Introduction: Cannabis continues to be a highly abused recreational drug. In addition, the increasing number of states legalizing it for medical use, combined with the trend towards legalization for recreational purposes means than analytical methods for the quantification of of Δ-9-tetrahydrocannabinol (THC), its metabolites and related cannabinoids continue to be necessary. Among drugs of abuse, natural cannabinoids present some unique analytical challenges. Excreted THC and related compounds are highly glucuronidated, requiring efficient deconjugation before analysis. In addition, the highly hydrophobic nature of natural cannabinoids makes them exceptionally susceptible to loss via non-specific binding, meaning that care must be taken with sample handling and processing of prepared extracts. Finally, matrix effects can be a challenge to control for these compounds, and can vary significantly in different biological matrices.

This work uses a novel reversed-phase solid phase extraction (SPE) sorbent, Oasis PRiME HLB, which has been developed to enable simpler and faster SPE protocols, while at the same time generating cleaner extracts than other sample preparation methods. 3 step load-wash-elute SPE protocols, eliminating conditioning and equilibration, were successfully employed to extract THC, OH-THC and COOH-THC from multiple matrices, including plasma, oral fluid (OF), whole blood and urine. This method details the extraction and analysis of these compounds using Oasis PRiME HLB in a µElution format, followed by direct analysis by UPLC/MS/MS. Specific modifications to SPE protocols or chromatography have been detailed to optimize the method for the various matrices.

Objective: This work details the systematic troubleshooting and optimization of SPE approaches with different biological matrices to achieve robust and consistent results.

Methods: For all 4 biological matrices, Oasis PRiME HLB µElution plates were used. All samples were pretreated as appropriate for the different matrices. The pretreated samples were then directly loaded to the µElution plate with a simple 3-step load-wash-elute protocol without condition/equilibration. The final eluates were diluted with water for direct LC/MS/MS injection without evaporation/reconstitution.

Results: Excellent recoveries (all greater than 80%, except one THC in urine), reproducibility (all in single digit) and matrix effects (all <20%) were obtained with the simplified protocol for all four biological matrices. UPLC/MS/MS analysis was rapid and highly consistent, with all analytes eluting within 3 minutes. Calibration curves were linear from 0.5-100 ng/mL with R2 values of > 0.99. Quality control results for all analytes in the four matrices were accurate and precise. All quality control results (n=6) had accuracies within 12% of expected concentrations and %RSDs of <10%. In each case, all FDA recommendations for accuracy, precision, linearity and analytical sensitivity were met for validated methods. With every matrix, proper sample preparation and column selection were discussed to get optimized performance.

One key advantage for Oasis PRiME HLB in SPE is its ability to remove phospholipids, thus creating a much cleaner extract. More than 90% of phospholipids were removed from the whole blood and plasma samples compared to protein precipitation (PPT) including those coeluting with the analytes of interest.

Conclusion: This application highlights the quantification THC and its metabolites in multiple biological matrixes with Oasis PRiME HLB. The consistent recoveries (single digit in variability) and low matrix effects (all <20%) demonstrated method reproducibility and robustness. The µElution format enabled the direct injection of extracts without evaporation or reconstitution, minimizing the risk of nonspecific binding. The SPE methods also eliminated phospholipids from samples, avoiding coelution with analytes of interest, and resulting in decreased matrix effects. Specific optimizations were necessary for each sample matrix to get high and consistent recoveries, low matrix effects, linear calibration curves, and accurate and precise quality control results.

Keywords: THCs, Oasis PRiME HLB, SPE

P08

Novel Automated Sample Preparation and LC/MS Analysis of Multi-Class Drugs

Dan Menasco*[1], Bruce Kempf[1], Elena Gairloch[1], Michael Roberts[2], Lee Williams[3]; [1]Biotage, Charlotte, NC, [2]Shimadzu Scientific Instruments, RTP, NC, [3]Biotage GB Limited, Dyffryn Business Park, Cardiff, UK

Background/Introduction: Investigative efforts to prepare, identify, and quantify, medications used to manage chronic pain and their respective metabolites in biological specimens have increased dramatically over the past decade. Although treating patients undergoing pain management is a rigorous and complex process, sentinel monitoring of their wellbeing is paramount. Nonetheless, the prescriptive augmentation of opiates, amphetamines, benzodiazepines, medicinal cannabinoids, and sympathomimetics, have engendered patient's comorbidities, which can result in unfavorable health outcomes, amplify the complexity of their clinical management, and increase health their care costs. The expansion of the aforementioned prescriptions have therefore increased the need, and in some cases necessitated, the direct and routine screening of multiple drug classes under various biological matrices.

Objective: To meet this challenge, a large multi-drug panel was developed to address both the complexity surrounding patient monitoring and the high throughput workflow associated with multiple patient matrices. Herein, we demonstrate the utility of a multi-drug panel using the Extrahera™ automated sample prep workstation with the Shimadzu 8060 LC/MS platform.

Method: Extractions were evaluated using both EVOLUTE® EXPRESS ABN and ISOLUTE® SLE+ 400 µL fixed well plates, respectively. Sample pretreatment and processing was performed on the Biotage® Extrahera™ sample preparation workstation configured with a 96-well positive pressure head for 96 well fixed plates. All samples were analyzed using a Shimadzu 8060 mass spectrometer under polarity switching conditions coupled to a Shimadzu Nexera 2 LC system. Compounds were identified by their experimentally determined MRM transitions and chromatographed using a 2.1 x 100 mm, 2.7 □m Restek Raptor Biphenyl column under 0.1% aqueous formic acid and methanol.

Result: Both supported liquid extraction (SLE) and solid phase extraction (SPE) protocols were developed for multiple drugs (opiates, amphetamines, benzodiazepines, medicinal cannabinoids, and sympathomimetics) and their corresponding internal standards on the Extrahera™ sample preparation workstation based on previous results with SLE and SPE. Sample and solvent volume, pipetting parameters, and flow characteristics were optimized using the Extrahera™ for each biological matrix analyzed. Chromatographic and mass spectrometric analyses showed excellent linearity and correlation ($r^2 > 0.99$) for all drugs analyzed which ultimately yielded excellent precision and accuracy over the therapeutic range of each analyte and at its LLOQ.

Conclusion/Discussion: Here we have shown that the combination of the Biotage® Extrahera™ sample preparation workstation and Shimadzu 8060 LC/MS system provide a facile platform for implementing a large therapeutic drug panel. Facile and robust sample preparation allowed for reproducible results and analyte recoveries while rapid mass spectrometric detection decreased run time and provided highly sensitive and accurate detection of drugs in multiple biological matrices.

Keywords: Automated Sample Preparation, Polarity-Switching, LC/MS

P09

LC/MS/MS Analysis of Phytocannabinoids and their Metabolites in Urine Using Various Sample Preparation Techniques

Rory M. Doyle, **Kristine Van Natta***, Thermo Fisher Sceintific, Inc, Somerset, NJ

Background/Introduction: Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC/MS/MS) is suited for rapid analysis of multiple analytes. A highly sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of phytocannabinoids and their metabolites that include- cannabidiol (CBD), cannabidiolic acid (CBDA), cannabinol (CBN), cannabinolic acid (CBNA), cannabidivarin (CBDV), cannabidivarinic acid (CBDVA), cannabigerol (CBG), cannanigerolic acid (CBGA), cannabichromene (CBC), tetrahydrocannabinol (THC), tetrahydrocannabinolic acid (THC-A), 11-hydroxy-delta9-THC (OH-THC), 11-nor-9-carboxy-delta9-THC (COOH-THC), tetrahydrocannabivarin (THCBV), cannabicitran (CBCT) and cannabinodiol (CBND) by QQQ. Various sample preparation techniques were developed in urine that includes dilute and shoot, liquid-liquid extract and solid-phase extraction. One dimensional (1D) chromatographic configurations achieved the required sensitivity and is capable of quantitating the analytes over their relevant dynamic range.

Method: A Thermo Fisher Endura in positive and negative electrospray mode and an UltiMate 3000 HPLC system were utilized for this analysis. 100 uL of human urine was used for the analysis of the various drug and metabolites. Various columns were evaluated and an Accucore C18 was used with a water:acetonitrile mixture containing 5 mM ammonium acetate gradient achieved baseline chromatographic separation in an approximately 5 minute run time. Quantitative analysis was performed using scheduled reaction monitoring (SRM) transition pairs for each analyte and internal standard in positive and negative mode and accuracy of the method was verified using reference materials from UTAK and human samples.

Result: Good linearity and reproducibility were obtained across the dynamic range of the drugs with a coefficient of determination R^2>0.995 for all drugs in the various matrices. The initial lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined as shown in the table and excellent reproducibility was observed for all compounds (CV < 15%) in all matrices.

Compound (ng/ml)	LOD Dilution	LOD Dilution	LOD LLE	LOQ LLE	LOQ SPE	LOQ SPE
THC	1	2.5	0.25	2.5	0.25	0.5
OH-THC	1	2.5	0.5	2.5	0.5	1
COOH-THC	0.5	2.5	0.25	1	0.25	1
CBC	5	10	2.5	10	2.5	5
CBD	2.5	5	1	2.5	1	2.5
CBDA	1	2.5	1	2.5	5	2.5
CBDV	1	2.5	0.5	1	0.5	1
CBG	0.5	2.5	0.5	1	0.5	1
CBGA	0.5	2.5	0.5	2.5	0.5	2.5
CBN	0.5	2.5	0.25	1	0.25	1
TCBDV	1	2.5	0.5	1	0.5	1

Conclusion/Discussion: A sensitive, simple, specific and accurate liquid chromatography QQQ mass spectrometry method was developed and verified for the simultaneous measurement of phytocannabinoids and their metabolites in urine.

Keywords: Phytocannabinoids, Urine Analysis, LC-MS/MS

P10

Novel Workflows Using a QTOF MS for Targeted and Non-Targeted Forensic Applications

Adrian Taylor*[1], Xiang He[2], Alexandre Wang[2]; [1]SCIEX, Concord, ON, Canada, [2]SCIEX, Redwood City, CA

Background/Introduction: Using accurate mass and high resolution information from TOF-MS and TOF-MS/MS, acquired on a Quadrupole Time-of-Flight (QTOF) mass spectrometer, allows for simultaneous highly specific targeted quantitation and non-targeted screening. Here we describe a new benchtop QTOF system with revolutionary N-geometry designed flight path and new, intuitive software for easy adoption of accurate mass technology to forensic testing.

Objective: Using this system we investigated several LC and novel MS workflows to maximize capturing all required information, in the shortest timeframe, that will allow for high level confident compound identification and quantification from blood or urine samples.

Methods: Urine samples were diluted 10-fold, blood samples underwent a protein crash. HPLC separation used a reverse-phase column, 30°C, investigating 6.0 and 2.0 and minute gradients. SCIEX X500R QTOF mass spectrometer with SCIEX OS software was used for acquisition and data processing: 1) TOF-MS survey scan with Information Dependent Acquisition (IDA)-triggering up to 16 product ion scans 2) MRM^{HR} or 3) SWATH® acquisition with variable Q1 isolation windows.

Results/Discussion: For positive identification purposes, the availability of MS/MS information was required in this study to perform library searching. Excellent true positive rates were accomplished using the 6 minute method at the compound cutoff concentrations. Acquiring MS/MS data allowed avoiding a false positive result in a donor sample, by not relying only on accurate mass, isotopic pattern and retention time. Simultaneous confirmation and concentration determination of each identified compound was also performed. Linearity on the new QTOF system for the majority of compounds tested was excellent with stable mass accuracy for analytes at all concentrations in the calibration curve of less than 1 ppm for the majority of compounds and less than 3 ppm for all compounds. Quantification performance and mass accuracy were evaluated using the faster LC gradients. At the cutoff concentration levels the library matching was successful but lower positive rates was achieved compared to the longer LC gradients, therefore the positive identification criteria needs to be modified. We will show that quantitation using MRM^{HR} and SWATH approach provides improved detection limits over the accurate mass of the precursor in TOF-MS scan mode and also allows for ion ratio confirmations.

Conclusion: A fast, sensitive and selective workflow was developed for forensic toxicological compound screening using the new X500R QTOF system.

Keywords: Mass Spectrometry, QTOF, Targeted and Non-Targeted Analysis

P11

Rapid and Sensitive Analysis of a 93-Compound Forensic Panel in Urine with a Hybrid Triple Quadrupole/Linear Ion Trap Mass Spectrometer

Adrian Taylor*[1], Michael Jarvis[1], Xiang He[2], Casey Burrows[2], Alexandre Wang[2]; [1]SCIEX, Concord, ON, Canada, [2]SCIEX, Redwood City, CA

Background/Introduction: (For research use only, not for use in diagnostic procedures). In this study we present a rapid, robust and sensitive analysis of a comprehensive forensic compound panel with 93compounds in human urine using QTRAP®/Triple Quadrupole 4500 LC/MS/MS system. The panel includes analysis of parents and metabolites of amphetamines, opiates, benzodiazepines, barbiturates, cannabinoids, cathinones, cocaine, carbamates, alkaloids, antidepressants, muscle relaxants and analgesics. The presence of several barbiturates and THC-COOH in the panel, which ionize better in negative mode, warranted the implementation of polarity switching. Due to a high number of total MRM transitions (212 MRMs including those of internal standards) and a short LC runtime (6.5 min), a newly optimized Scheduled MRM algorithm is used.

Objective: Develop a rapid, single LC-MS/MS method to effectively quantitate a comprehensive panel of compounds of differing chemistries.

Methods: Blank human urine was used to prepare calibrators. Four levels of calibrators were prepared. The concentration ratio between these calibrators was always (in descending order): 20:6:2:1. Urine sample was hydrolyzed at 50 degrees centigrade using IMCSzyme. After hydrolysis, methanol and water were added to the mixture prior to LC-MS/MS analysis. HPLC separation was performed with SCIEX ExionLC™ AC HPLC system. Phenomenex Kinetex Phenyl-hexyl column (50 × 4.6 mm, 2.6 µm), were used. Mobile phase A (MPA) was 5mM ammonium formate in water and mobile phase B (MPB) was 0.05 %formic acid in methanol. The LC flowrate was 1 mL/min and the LC runtime was a 6.5 min gradient. Injection volume was 5 µL. Data acquisition was done with Analyst 1.6.3. Target scan time was 0.2 sec and 0.05 sec for positive and negative modes, respectively. Pause times between MRM transitions were set to 5 msec. Detection window was 20 sec for both ionization modes. Quantitation was performed with MultiQuant software 3.0.

Result: We achieved fast separation of the commonly targeted isobaric compounds despite the short LC runtime. We achieved a minimum of 10 data points across the LC peak; the majority of the MRM transitions had over 15 or more data points. We observed a strong MRM signal at the lowest calibrator level (1 ng/mL), suggesting the possibility of reaching even lower LOQ with a mere 0.5 pg on column. Excellent linearity (r2 values >0.99) and reproducibility (<10% CVs) was observed throughout the dynamic range assessed in this effort. It was essential to utilize polarity switching to accommodate more than 200 MRMs within the one short data acquisition method.

Conclusion/Discussion: A rapid and sensitive method for the LC-MS/MS analysis of 93- compound forensic panel in human urine was developed on the SCIEX ExionLC™ AC HPLC and QTRAP®/Triple Quadrupole 4500 LC/MS/MS system. This method utilized a dilute-and-shoot procedure, Scheduled MRM algorithm and polarity switching. Excellent linearity and precision were observed for all the compounds in the listed calibration range.

Keywords: Mass Spectrometry, Polarity Switching, Scheduled MRM

P12

Rapid LCMS Method Development for Multiple Drug Classes by Using a New Four-Channel HPLC

Pengxiang Yang*, Kristine Van Natta, Christopher Elicone; Thermo Fisher Scientific, San Jose, CA

Background/Introduction: With regard to LC/MS analysis being used in clinical research, LC/MS method development is crucial but time-consuming due to the complexity of compounds. Developing LC methods for multiple drug classes provides greater challenges, in part, due to the consideration of various HPLC columns, various mobile phases and different gradients that can be used. By using a new four-channel LC system, the LC method development can be done rapidly and efficiently. In this poster, four different LC methods were developed and optimized rapidly for four classes of compounds (anticonvulsants, antidepressants, antipsychotics, and antiarrhythmics) in plasma.

Methods: Published sources as well as chemical properties of target compounds (anticonvulsants, antidepressants, antipsychotics, and antiarrhythmics) were considered in order to select four UPLC/HPLC columns. Four different columns (Thermo Scientific™ Hypersil™ GOLD Phenyl, 1.9 µm, 50x2.1 mm, Hypersil™ GOLD, 1.9 µm, 50 x 2.1 mm, Hypersil™ GOLD aQ, 1.9 µm, 50 x 2.1 mm and Accucore™ PFP, 2.6 µm, 50 x 2.1 mm) were installed on a pre-production Thermo Scientific Prelude LX-4 MD™ HPLC which has four independent LC channels. Compounds were detected on a prototype Thermo Scientific™ Endura MD™ triple quadrupole mass spectrometer and two selected-reaction monitoring (SRM) transitions were monitored for each analyte to obtain ion ratio confirmation (IRC). In this study, stable isotope-labeled internal standards of most compounds were used. After the rapid LCMS method development, calibration standards and quality controls (QC) of four classes of drug compounds in plasma were injected to challenge the method. Precisions and accuracies were evaluated by analyzing a single calibration curve along with six replicates of QCs on three successive days. Analytical stability of the system over time was evaluated by quantitating QCs using a calibration curve that was run two days earlier with using stable-labeled internal standards.

Result: Three UPLC columns and one HPLC columns were selected to separate four classes of drug compounds (anticonvulsants, antidepressants, antipsychotics, and antiarrhythmics). Three linear gradients with different initial %MP-B were programmed as three test methods to check the four UPLC/HPLC columns' performance in a single batch (four samples, four injection channels, three 5-minute gradients, totaling 96 injections and 8 hours instrument time). We quickly confirmed the best UPLC/HPLC column for each drug class based on the baseline separation and peak shape of each compounds. The batch data provided enough information, such as elution time, peak shape, peak tailing and baseline separation, to adjust the LC gradient in four different LC methods. By using the new four-channel LC system, we completed LC method development for four classes of drug compounds (24 compounds) within 24 hours. Calibration standards of 24 compounds in plasma were analyzed with the developed methods. Accuracies calculated by comparing the measured value of QCs to the theoretical value were better than 84% and precisions expressed as %RSD were better than 14.7% across all compounds and all concentrations. QCs for anticonvulsants quantitated using a calibration curve from two days earlier still gave accurate results greater than 87%. When a set of samples (calibrators, QCs, and blanks) for each compound class were run in serial, the entire analysis time was 23:04 (hh:mm). When multi-channeling was enabled, the time dropped to 08:26 (hh:mm), a time savings of 63%.

Conclusion/Discussion: We were able to develop four different LC methods for 4 separate compound classes rapidly with one HPLC-MS system. All compounds showed good accuracy and precision over the course of evaluation. The system showed good analytical stability in its ability to give accurate results from the same calibration curve over several days. Furthermore, implementation of multi-channeling saved 63% runtime over running the same methods in serial.

Keywords: Four-Channel HPLC, Multiple Drug Classes, LCMS

P13

Analysis of Δ^9-Tetrahydrocannabinol and Its Two Main Metabolites in Whole Blood Using Automated Dispersive Pipette Extraction and LC-MS/MS

Kaylee R. Mastrianni*[1], William E. Brewer[1,2], Dustin W. Smith[3], Wendy Bell[3], Stephen L. Morgan; [1]Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC; [2]DPX Technologies, LLC, Columbia, SC; [3]South Carolina Law Enforcement Division, Columbia, SC

Introduction: Marijuana is the most widely abused drug in the US. As marijuana continues to be legalized, the need for easy, fast, and sensitive quantitative methods for the detection of Δ^9-tetrahydrocannabinol and associated metabolites increases. Blood is the traditional matrix for determining impairment for suspected DUID cases. The complex nature of blood can make drug determinations with LC-MS/MS difficult due to the presence of matrix causing analyte ion suppression/enhancement, known as matrix effects. Therefore, sample preparation is a necessity. Previously published work generally requires large sample volumes and complex sample preparation in order to achieve essential sensitivity.

Objective: Our aim was to develop a method that minimizes sample volume and automates a fast and easy solid phase extraction procedure to obtain a sensitive quantitation of Δ^9-Tetrahydrocannabinol (THC), 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) in whole blood using LC-MS/MS with method validation according to the SWGTOX guidelines.

Methods: *Sample preparation:* Aliquots of 100 uL of each sample were transferred to a 2 mL micro centrifuge tube. Internal standard in methanol was added and the tubes were vortex mixed. Acetonitrile (300 uL) was added and the tubes were vortexed and centrifuged. The supernatant was transferred to a well plate, which was then placed on the Hamilton NIMBUS96 system for the automated solid phase extraction procedure. After the addition of 50 uL of 0.1M formic acid to the sample supernatant, DPX WAX-S tips are used to aspirate and dispense the sample solution three times. This allows for extraction of matrix and subsequent partitioning of the acetonitrile and the aqueous phase. An aliquot of 100 uL of the supernatant (acetonitrile layer) is transferred to a clean well plate, which is transferred to the LC-MS/MS for injection.

Results & Discussion: *Method Validation:* The method was validated according to SWGTOX guidelines. Therefore, the method was evaluated for bias, precision, carryover, linearity, interferences, matrix effects, and limit of detection and quantitation. The method was very accurate and precise. The bias of the assay did not exceed 6% for any analyte over each concentration. The precision (coefficient of variation, %) did not exceed 11% for any analyte over each concentration for both within and between run variations. The calibration models were linear, each correlation coefficient was greater than 0.99 with a 1/x weighting. The method was free from carryover at 50 ng/mL, the highest calibrator. Matrix effects were negligible for 11-OH-THC and THC-COOH, but THC experienced 31% ion suppression at 5 ng/mL. The extraction efficiency of the automated SPE was $\geq$ 93%. Limits of detection were calculated to be 0.25-0.62 ng/mL. Limits of quantitation were 0.75-1.8 ng/mL.*Interlaboratory Comparison with SC State Law Enforcement Division:*A successful patient sample comparison was completed with the South Carolina Law Enforcement Division (SLED). Twenty-eight patient samples that were previously analyzed by SLED's validated method were evaluated with this method. Each patient sample was analyzed in triplicate using the method described above. The correlation coefficient for the comparison of 11-OH-THC positive samples was 0.9965 (n=3), THC-COOH was 0.9836 (n=25), and THC was 0.996 (n=16). The percent difference in calculated results did not exceed 20%. The coefficient of variation of the triplicate extractions of patient samples did not exceed 15%.

Conclusion: The method presented herein provides an easy, semi-automated approach to analyzing THC, THC-COOH, and 11-OH-THC in whole blood using LC-MS/MS. Protein precipitation is performed manually with the addition of acetonitrile and vortex/centrifugation, while solid phase extraction is fully automated on a Hamilton NIMBUS96 platform. The method is robust, with high precision and accuracy.

Keywords: Tetrahydrocannabinol, DPX, Blood

P14

Development of a Lateral Flow Immunoassay Capable of Detecting Ochratoxin A

David Osselton*, Georgina Ross, Dene Baldwin; Department of Archaeology, Anthropology and Forensic Science, Faculty of Science and Technology, Bournemouth University, Fern Barrow, Poole, Dorset, UK

Background/Introduction: Mycotoxins are secondary, highly toxic metabolites of several filamentous moulds. These toxins are prevalent in agricultural commodities. Ochratoxin A (OTA) is one such mycotoxin produced by many species of *Aspergillus* and *Penicillium* and has been recognised as a common contaminant of cereal crops intended for human and animal consumption. As OTA is genotoxic, hepatotoxic, neurotoxic, immunotoxic, teratogenic and has been recognised by the International Agency for Research on Cancer as a possible group 2B human carcinogen, it is critical to limit the quantity of OTA present in food sources.

Objective: The objective of this research was to design and develop a lateral flow immunoassay capable of detecting minute quantities of Ochratoxin A. It is necessary for rapid assays which can be used to rapidly, visually detect OTA contamination under non-laboratory conditions which are in compliance with the European tolerable daily intake levels.

Methods: A lateral flow immunoassay has been developed using a monoclonal antibody against OTA labelled with gold nanoparticles as the visual detector. The optimum conditions for conjugation of 40nm colloidal gold to OTA-MAb were established and implemented at the BioHub, Birmingham University, UK in collaboration with Abingdon Health Ltd. (for reasons of commercial confidentiality, further details are not provided within this poster). The reagents were deposited by spraying onto nitrocellulose membrane by spraying using ZX1000B BioDot Dispenser. Ochratoxin A-Conjugated-Bovine Serum Albumin (OTA-BSA) was immobilised as the test line; the control line was sprayed with Sheep-anti-mouse Antibody (SaMAb) and the visual detector was OTA-MAb conjugated to colloidal gold. Three separate concentrations of OTA-BSA (test line) were sprayed onto separate strips for testing for optimum conditions for OTA detection. The strips comprised a nitrocellulose reaction membrane, a glass fibre sample and conjugation pad (optional), together with an absorbent pad, backed with laminate plastic backing. The dipsticks were cut into 5mm strips using BioDot CM4000 guillotine and sealed in foil pouches with desiccant silica beads.

Result: The dipsticks were tested in blank cereal samples (Extracted with 70:30 methanol/water) spiked with various concentrations of Ochratoxin A standard (10µg/ml in acetonitrile; Sigma Aldrich) and various buffers to establish the visual limit of detection. The limit of detection for this assay was defined as the lowest concentration that gave two red lines (control and test line) for a sample not containing OTA and one red line (control line) for a sample spiked with OTA. The working visual limit detection of this assay currently is 100ng/mL of OTA, this is in accordance with European tolerable daily intake limits.

Conclusion/Discussion: It is crucial for the development of sensitive lateral flow assays for the detection of a range of food contaminants in order to avoid health implications for humans and animals. Mycotoxins compose a significant percentage of food contaminants so it is necessary to be able to detect and minimise OTA contamination to comply with maximum admissible levels of these mycotoxins. The OTA detection dipsticks provide a visually deterministic device which can distinguish between OTA presence and absence in samples. The dipsticks were manufactured in such a way as to allow for future multiplexing of the device to allow for simultaneous detection of multiple mycotoxins in a singular sample.

Keywords: Ochratoxin A, Immunoassay, Lateral-Flow

P15

Quantitative Determination of Paracetamol (Acetaminophen) in Human Whole Blood and Vitreous Humour *in vivo* from Patients undergoing Vitrectomy Surgery

Stephanie Farrant[1], Andrew Morris[2], Nicola Jones[1], **M. David Osselton*[1]**, Shane McCabe[2], DJ Dagleish[2], Avinash Aswath[2]; [1]Faculty of Science and Technology, Bournemouth University, Talbot Campus, Fern Barrow, Poole, Dorset, UK, [2]Royal Bournemouth Hospital, Castle Lane East, Bournemouth, UK

Background/Introduction: This presentation describes the findings of an investigation to determine the distribution of paracetamol between blood and vitreous humour in living patients undergoing vitrectomy surgical procedures. Post-mortem toxicology is routinely used to determine the contribution of drugs or poisons to an individual's death however, interpreting the results of post-mortem analysis is often challenging due to uncertainties regarding the disposition of drugs within the body. Post-mortem drug concentrations in blood do not always reflect antemortem drug concentrations as a consequence of post-mortem redistribution. Because drug concentrations can be significantly affected by post mortem change alternative matrices such as the vitreous humour have been investigated to support interpretation. Although blood is currently the preferred matrix for the determination of drug concentrations at the time of death, this may not be truly representative when applied to cases in which there is a time delay between death and sampling. It has been well documented that many drugs enter into the vitreous humour following drug consumption and the ease with which vitreous fluid may be analysed has led toxicologists to investigate vitreous as an alternative matrix for toxicological investigation. This study uniquely provides ratios between blood and vitreous where samples have not been subject to post-mortem variation.

Objective: Although vitreous humour offers many advantages as a specimen for the forensic toxicologist there is a lack of knowledge regarding drug movement and kinetics from the circulating blood into the eye. In this study, *in vivo* paracetamol concentrations were measured in vitreous humour and venous blood collected from patients undergoing vitrectomy surgery at Bournemouth Hospital (n=17). Administration of paracetamol was either via an oral pre-medication routine approximately 1-2 hours before the procedure or via intravenous (IV) infusion 10-20 minute's pre-operative. Blood and vitreous samples were collected simultaneously in order to determine a ratio between the two samples.

Methods: All samples were extracted using a modified LLE procedure from that outlined by Simonsen et al (2010). Chromatography was undertaken using a Perkin-Elmer series 200 HPLC system with an autosampler, binary pump, fixed wavelength UV detector, vacuum degasser and a C8 column (5 µm). The mobile phase used consisted of HPLC grade acetonitrile: water (1:3, v/v) adjusted to pH 7 using phosphoric acid.

Result: Results showed that paracetamol can be detected in vitreous humour samples following the administration of a 1g dose either orally or via infusion. Table 1 shows the relative concentrations found in both specimens and their respective vitreous: blood (V:B) ratios (n=17 patients). The results show distinct differences in drug ratios depending on the route of administration which may also reflect the time taken for the drug to enter the vitreous.

Conclusion/Discussion: Paracetamol enters the vitreous humour following oral and IV dosing. Concentrations were significantly higher in blood than in the vitreous humour with an overall mean vitreous: blood ratio of 0.21. The range of paracetamol concentrations were 3.74-76.07 µg/ml and 0.31-6.54 µg/ml in blood and vitreous humour respectively. The route of paracetamol administration greatly influenced the V:B ratios. Patients administered paracetamol orally had a mean V:B ratio of 0.3729 compared to the IV mean V:B ratio of 0.083. It is highly likely that the V:B ratio is greatly affected by many factors, especially the time between drug administration and sample collection. The results also demonstrate that paracetamol is capable of entering vitreous fluid rapidly following entry into the circulating blood. OTA detection dipsticks provide a visually deterministic device which can distinguish between OTA presence and absence in samples. The dipsticks were manufactured in such a way as to allow for future multiplexing of the device to allow for simultaneous detection of multiple mycotoxins in a singular sample.

Table 1 – Paracetamol concentrations found in blood and vitreous humour samples and their respective V:B ratios.

Route of Administration	Vitreous Concentration	Mean Blood Concentration	V:B ratio
Oral	4.03	10.24	0.39
Oral	3.81	15.64	0.24
Oral	3.42	10.99	0.31
Oral	4.89	20.53	0.24
Oral	6.54	10.89	0.6
Oral	2.37	3.74	0.63
Oral	2.56	12.94	0.2
Mean	**3.95 (2.37-6.54)**	**12.14 (3.74-20.53)**	**0.37 (0.2-0.63)**
IV	0.52	76.07	0.01
IV	0.63	36.2	0.02
IV	0.31	29.31	0.01
IV	2.1	47.08	0.04
IV	2.88	48.72	0.06
IV	1.26	44.66	0.03
IV	2.97	31.13	0.1
IV	2.89	49.03	0.06
IV	1.33	23.03	0.06
IV	2.47	30.62	0.08
Mean	**1.74 (0.31-2.89)**	**41.59(23.03-76.07)**	**0.08 (0.01-0.1)**

Keywords: Acetaminophen, Vitreous: Blood Ratio, Liquid-Liquid Extraction (LLE)

P16

Evaluation of the Use of Dual Laser Handheld Raman Spectroscopy for the Identification of Counterfeit Herbal Life-Style Products Purchased Worldwide

Dimitra Florou, Sulaf Assi, **David Osselton***; Department of Archaeology, Anthropology and Forensic Science, Bournemouth University, Fern Barrow, Talbot Campus, Poole, Dorset, UK

Background/Introduction: The last few decades have witnessed a change in the overall use of medicines. This involved the use of medicinal and non-medicinal products for overall improvement of the lifestyle of individuals via enhancing the image, activity and performance. Specifically, herbal lifestyle products are often advertised as safe and effective with minimum adverse effects. However, often these products are counterfeited by synthetic adulterants which promote their activity. For instance, the presence of phencyclidine, which is an illicit hallucinogen have been found in counterfeit Chinese herbal weight loss products. As these products could be encountered anywhere, rapid methods are crucial for their identification. Handheld Raman spectroscopy is rapid and non-destructive technique used in authentication of medicines. Yet, one major disadvantage of handheld Raman is the fluorescence of Raman inactive material (such as herbal materials). The use of sequentially shifted excitation (SSE) algorithm would overcome this disadvantage.

Objective: To evaluate the use of the dual laser handheld Raman spectrometer equipped with SSE algorithm for the identification of herbal life-style products and investigation of the manufacturing source.

Methods: A total of 50 lifestyle products were purchased from five countries and the Internet worldwide. The products were of five main classes including: antidepressants, herbal sleep aids, memory enhancers, sexual stimulants and weight loss. Additionally, the constituents of the products (where known) were purchased from suppliers. Powder mixtures of raw materials and products were also prepared. Products were measured 'as received' using the Bruker BRAVO handheld Raman spectrometer equipped with dual laser. The raw Raman spectra of the products were analysed using both the OPUS software (v 7.7) and Microsoft Excel 2010. Data treatment was conducted using spectral comparison, cluster analysis and correlation methods.

Result: The Raman activity of the products depended on their main constituents as well as the quantity of constituents present in the preparation. Hence, when a Raman active material was present in a high concentration, above 10% m/m, the product showed spectral features corresponding to the aforementioned material [1]. Products containing high concentration of valerian extracts showed significant spectral features corresponding to methyl isovalerate, phenyl isovalerate and valeric acid. In this case, the spectra of Raman active products were evaluated using clustering algorithms. The clustering of the products corresponded to their manufacturing source. On the other hand, products that contained mainly herbal material did not show any Raman activity. This was useful when identifying an adulterated herbal material. In this instance, when a synthetic derivative was encountered in the herbal material, a significant Raman spectrum was observed corresponding to the synthetic substance. Thus, caffeine was encountered in counterfeit memory enhancers and sildenafil was detected in counterfeit herbal sexual stimulants (Figure 1). Subsequently, mixtures of synthetic substances and products were prepared to evaluate the minimum concentration of synthetic substance detected. In this respect, the lowest concentration of a synthetic substance detected in a counterfeit herbal product was 10% m/m.

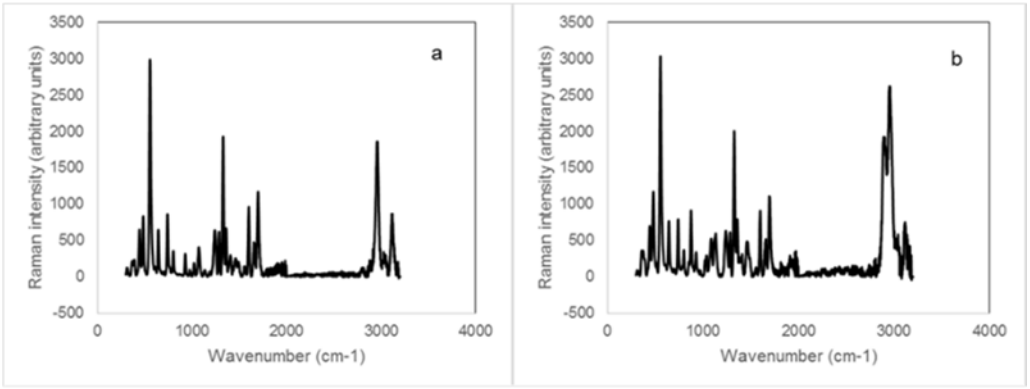


Figure 1: Raman spectra of (a) pure caffeine and (b) products with 31% caffeine measured using the Bruker BRAVO handheld Raman spectrometer equipped with dual laser.

Conclusion/Discussion: The handheld Raman spectrometer has been shown to be effective in characterising herbal products provided they contained high concentration of Raman active material (>10% m/m). Additionally, Raman spectroscopy has been demonstrated to be efficient in identifying adulterated Raman inactive products. The application of Surface Enhanced Raman Spectroscopy (SERS) will enable an increase in the sensitivity of the method to detect low concentration of adulterants in herbal products.

Acknowledgement: Bruker for the BRAVO Raman spectrometer.

References: [1] Assi, S., Watt, R. and Moffat, T., 2011. Authentication of medicines using Raman spectroscopy. European Pharmaceutical Review, February 16.

Keywords: Handheld Raman, Counterfeit Herbal Lifestyle Products, Sequentially Shifted Excitation.

P17
Streamlining Sample Preparation with Second Generation Enzymes

Kavinda De Silva*[1], Ray Kwan[1], Siobhan McKenny[1], Manuel Rozas[2]; [1]Molecular TestingLabs, Vancouver, WA, [2]KURA Biotec, Puerto Varas, Chile

Background/Introduction: Even though enzymatic hydrolysis is the optimum approach used for sample preparation in analytical toxicology, some drawbacks of this technique includes long incubation times, poor analyte recovery and short column lifetime due to high concentration of proteins loaded onto the HPLC column. We tested the first generation beta-glucuronidase currently used in our laboratory (DR2102 from Campbell Scientific) against a beta-glucuronidase also isolated from *Haliotis rufescens* (BG100® from Kura Biotec) and a second generation enzyme (BGTurbo™ also from Kura Biotec), assessing final analyte recovery and total protein concentration loaded onto HPLC column.

Objective: Compare hydrolysis efficiency and potential column lifetime of first and second generation beta-glucuronidase enzymes used for analyte quantification.

Methods: Blank urine was fortified with 300 ng of conjugated buprenorphine, codeine, hydromorphone, lorazepam, morphine, oxymorphone and THC-COOH glucuronides. The sample hydrolysis was performed, in triplicate, as follows: 50 µl of fortified urine, 25 µl of buffer, 20 µl of the internal standards and 10 µl (DR2102 and BG100®) or 20 µl (BGTurbo™). Incubation was carried out for 60 minutes at 62 °C for samples hydrolyzed with DR2102; 30 minutes at 68 °C for BG100®; and 15 minutes at 50 °C for BGTurbo™. Samples were then centrifuged at 24,000 rpm to reduce protein excess, diluted with 450 µl (95% water; 5% methanol) and loaded onto Biphenyl column (Phenomenex®) and analyzed using SCIEX 5500 QQQ LC-MS/MS. Total protein in the samples before and after centrifugation was measured in triplicate by absorption at 280 nm with NanoDrop Lite™(Thermo Fischer Scientific).

Result: Besides reducing the sample incubation time with second generation enzymes, table 1 shows that average analyte recovery was also improved in average by 16% in half the time for BG100® and 15% in one fourth of the time for BGTurbo™, when compared to hydrolysis with DR2102. BG100® was better for buprenorphine and oxymorphone recovery, while BGTurbo™ was particularly more reactive to codeine than to any other analyte. On the other side, the amount of protein remaining in the samples after centrifugation was decreased by 61 % for hydrolysis with BG100® and 81 % for BGTurbo™ compared with DR2102 (table 2). In this matter, preliminary results have shown a 4 fold increase in column lifetime by using second generation over first generation enzymes (data not shown). Interestingly, second generation enzymes show less remaining protein before centrifugation than samples hydrolyzed with DR2102 after centrifugation, suggesting that centrifugation step could be avoided with the new enzymes, improving even more the sample preparation setup.

Table 1. Analyte recovery obtained for the beta-glucuronidases tested.

	Analyte recovery (ng/mL)		
Analyte	DR2102 Control	BG100®	BGTurbo™
Buprenorphine	223.1	271.1	224.4
Codeine	159.4	173.8	243.2
Hydromorphone	176.8	196.0	196.2
Lorazepam	214.9	202.5	185.9
Morphine	174.5	190.9	198.1
Oxymorphone	155.8	189.0	169.4
THC-COOH	75.7	146.0	140.6
Average analyte recovery (% of recovery improvement)	168.6	195.6 (16 %)	193.9 (15 %)

Table 2. Total protein concentration before and after centrifugation.

Centrifugation	Total protein (mg/mL) DR2102 Control	BG100®	BGTurbo™
Before	1,788	0,554	0,103
After	0,725	0,280	0,085
(% of reduction of protein in sample)		(61 %)	(88 %)

Conclusion/Discussion: These new enzymes were able to reduce up to 4 times hydrolysis incubation, improve overall analyte recovery by up to 16 % and decrease total protein concentration of samples up to 88 %. All these improvements will help directly to extend column lifetime, as well as to improve both sample preparation efficiency and precision of the results obtained in analytical toxicology laboratories. A benchmark of authentic urine patient samples will be the next step to quantify the analytical benefits and operational savings that can be derived by second generation beta-glucuronidases.

Keywords: Sample Preparation, β-Glucuronidase, Enzymatic Hydrolysis

P18

Simultaneous Quantification of 64 Drugs and Metabolites in Human Urine Using Threshold Accurate Calibration Technique

Thomas G. Rosano*[1,2], Patrice Y. Ohouo[2], John J. LeQue[2], Michelle Wood[3]; [1]Forensic Toxicology Laboratory, Department of Pathology and Laboratory Medicine, Albany Medical Center Hospital and College, Albany, NY, [2]Clinical and Forensic Toxicology Laboratory, National Toxicology Center, Schuylerville, NY, [3]Health Sciences Organization, Waters Corporation, Wilmslow, UK

Background: Drug and metabolite (analyte) identification with quantification is an important analytical tool in forensic toxicology and current mass spectrometry methods traditionally employ stable isotope internal standardization (SI-IS) to achieve linear calibration. We have recently reported the use of a Threshold Accurate Calibration (TAC) technique employing dual specimen analysis, *i.e.,* with and without addition of a reference-analyte standard, for achieving threshold accurate identification in multi-drug urine screening. The new TAC-based screen achieved normalization of matrix effects without the need for SI-IS and quantitative accuracy was established at 50-150% of threshold concentration. This latest study aims to assess the application of the TAC approach over a wider quantitative range.

Objective: The aim of this study was to develop a method, based on the TAC principle and utilizing ultra-performance liquid chromatography (UPLC) in combination with tandem mass spectrometry (MS/MS), for the simultaneous detection and quantification of multiple analytes and to conduct studies that assess the precision and accuracy of the method for application in routine toxicology practice. The 64 drugs and metabolites were from the following drug classes: opiate, opioid, opioid antagonist, sedative (including benzodiazepines), amphetamine, designer amphetamine, cathinone, cocaine, hallucinogen and gabapentinoid.

Method: Dual aliquots (25 µL) of each calibrator, control and case specimen were transferred into adjacent positions within a 96-well plate prior to the addition, to one of the paired-aliquots, of a reference-analyte spike containing the 64 reference analytes at a concentration 1.3 times the upper limit of quantitation (ULQ). Injection volume precision was monitored by addition of methapyrilene to both aliquots. Following a one hour hydrolysis with purified β-glucuronidase at 55 ^{o}C, the reaction mixture was diluted 27-fold with starting mobile phase and directly analyzed by UPLC-MS/MS (Waters ACQUITY I-Class-Xevo TQD system). Chromatographic separation was achieved within 3 min with an ACQUITY UPLC BEH phenyl analytical column. The mass spectrometer was operated in positive electrospray and two transitions were used for all analytes, except tramadol and the *N*-desmethyltramadol metabolite where only a single transition was available. Resultant transition responses were exported into a custom Microsoft Excel template for TAC ratio calculation using the formula (ion area without analyte spike)/(ion area with analyte spike – ion area without analyte spike). A five-point weighted least squares regression of TAC ratio versus concentration was employed for assay calibration with transition ion ratio criteria, calibrator data reanalysis, injection precision determination and quality control assessment. Analyte-specific calibration ranges of 2.5-150, 6.25- 37.5 or 25-1500 ng/mL were used, with the lowest calibration range for analysis of fentanyl, norfentanyl, buprenorphine, norbuprenorphine, 6-acetylmorphine, mitragynine and hydroxymitragynine.

Results: Reagent optimization showed assay linearity and quantitative performance maximized with concentrations of the reference-analyte spiked at, or above, the concentration of the ULQ. The calibration model was linear for all 64 analytes with R^2 >0.99 and calibrator re-analysis within 20% of target. Quality control analysis at concentrations of 1.5, 4 and 48 times the lower limit of quantitation (LLQ) demonstrated precision within 20% and percent coefficient of variation mean (range) of 7.6 (2.6-19), 8.0 (3.9-14) and 9.3 (5.1-14), respectively. Bias for all quality control analyses averaged -5.5% with a range of -15 to 4.4%. Limit of detection (LOD) averaged 13 ng/mL (range: 1.6-25 ng/mL) with the LOD below the LLQ for all analytes. Method accuracy was determined by proficiency testing and cross-over testing with previously validated definitive confirmation methods. Excel template calculations assisted in data management for simultaneous quantification of 64 analytes in routine practice.

Conclusions/Discussion: A TAC technique has been developed for accurate multi-analyte quantification in human urine with rapid hydrolysis and direct injection of the diluted urine. The novel calibration technique achieved normalization of matrix effect without the use of stable-isotope internal standardization. Validation performance indicates applicability in routine forensic and clinical toxicology practice.

Keywords: Urine Drug Quantification, Threshold Accurate Calibration, UPLC-MS/MS

P19

Application of a New Fully Automated Rapid Screening System for the Detection of 20 Drugs of Abuse from One Sample of Oral Fluid

Joanne Darragh*, Victoria Anderson, Paul Vance, Sasha Cardwell, Danielle Doone, Jennifer Dicks, Aisling Speers, M. Elouard Benchikh, R. Ivan McConnell, S. Peter FitzGerald; Randox Toxicology Limited, Crumlin, Co Antrim, UK

Background/Introduction: Rapid drug screening can play a vital role in workplace drug testing particularly where safety is critical. The reduced time to first result could speed up the confirmation of an individual's impairment preventing any further risk to colleagues or the general public. Oral fluid provides an ideal matrix for testing in this environment with a quick, simple and non invasive collection method. Positive test results are indicative of recent drug use.

Objective: This study presents the application of a new rapid screening system Evidence MultiSTAT, which is based on biochip array technology, to high density immunoassay based detection of 20 drugs of abuse from a single oral fluid sample in under 20 minutes whilst maintaining sensitivity and accuracy

Methods: Simultaneous competitive chemiluminescent immunoassays on a biochip surface and applied to the Evidence MultiSTAT analyzer were employed. This fully automated system fully processes a self-contained cartridge containing all the components required for the immunoassay reactions and has the capacity to assess two biochips in under 20 minutes. Sampling 100µl of oral fluid against a cut-off sample, the results obtained are qualitative. Precision was determined by preparing and assessing samples at +50% of the cut off and -50% of the cut-off against a cut-off sample. The samples were analyzed twice per day for 10 days on 2 different analyzers giving a total of 40 replicates. The qualitative results were determined and presented as percentage agreement. Accuracy was determined as follows: 100 samples prepared in the Intercept I2 buffer were assessed (40 negative samples and 60 spiked samples spanning the cut-off). Each sample was ran against a cut-off sample, a qualitative result was determined for each of the 20 drug classes and the results presented as percentage agreement to a spiked value. In addition, 90 authentic oral fluid samples were collected using an Oral-Eze collection device for 6 of the drug classes present on the biochip array. Each sample was ran on the Evidence MultiSTAT analyzer against a cut-off sample. A qualitative result was determined for each of the 6 drug classes and the results presented as percentage agreement to LC/MS.

Result: The following drug classes were detected with the associated cut-off values: Fentanyl (1ng/mL), Ketamine (50ng/mL), LSD (1ng/mL), Methamphetamine (50ng/ml), Barbiturates (50ng/ml) , Benzodiazepines(10ng/mL) , Methadone (4ng/mL), Opiate (15ng/mL), PCP (5ng/mL), Benzoylecgonine (20ng/mL), Oxycodone (8ng/mL), Tramadol (4ng/mL) , Amphetamine (50ng/mL), THC (4ng/mL), Buprenorphine (1ng/mL), 6-MAM (2ng/mL) , JWH018 (15ng/mL), alpha PVP (2ng/mL), UR144 (20ng/mL). For the +50% and -50% cut-off samples analyzed across 2 analyzers, the precision evaluation showed a percentage agreement of 100% for all assays with the exception of PCP, alpha PVP and Buprenorphine which was 97.5% (39 out of 40 samples). Analysis of the 100 (60 spiked and 40 negative) Intercept I2 samples produced a percentage agreement of 100% for 10 of the assays assessed; the remaining 10 assays produced percentage agreements in the range of 94% to 99%. Analysis of authentic oral fluid samples produced a percentage agreement of 100% for Methamphetamine, 97.8% for Methadone, 94.4% for Benzodiazepine, 94.4% for Amphetamine, 91.1% for Opiate and 90% for 6-MAM.

Conclusion/Discussion: The data presented indicates that 20 drug classes can be screened in less than 20 minutes to yield reproducible and accurate qualitative results by using the fully automated Evidence MutiSTAT system. The cut-offs achieved are extremely sensitive and applicable for an oral fluid matrix. This reported new application, utilizing the biochip array technology, is an effective, quick and user friendly solution for oral fluid screening. This provides an ideal system to be used in workplace drug testing and in Forensic Laboratories looking for a quick turnaround.

Keywords: Oral Fluid, Biochip Array Technology, Fully Automated

P20

Screening of the Recommended Drugs Associated with Driving Under the Influence of Drugs from a Single Urine Sample using a Biochip Array

Laura Keery*, Christopher Stevenson, Rebecca Keenan, Joanne Darragh, R. Ivan McConnell, S. Peter FitzGerald; Randox Toxicology Limited, Crumlin, Co Antrim, UK

Background/Introduction: Drug impaired driving is becoming a major problem in the US and Worldwide. Drug detection involves initial screening of samples for drugs. For legal purposes, the screening procedure eliminates all negatives and positive results require confirmation using a confirmatory method. Biochip array technology (BAT) enables the simultaneous detection of multiple analytes from a single sample, which is advantageous to consolidate testing and to increase the screening capacity.

Objective: The aim of this study was to apply BAT to the simultaneous screening of drugs associated with Driving Under the Influence of Drugs (DUID) included in the two-tier approach based on recommendations for the toxicological investigation of drug-impaired driving and motor vehicle fatalities. Tier 1 consisted of the most prevalent drugs found in the US impaired driving population and should be the minimum testing to be completed in drug driving casework. Tier 2 drugs being less frequently encountered, with regional significance and/or beyond the routine analytical capabilities of some laboratories. The application reported here allows the screening of Tier 1 and Tier 2 drugs from a single urine sample using a biochip array.

Methods: Competitive chemiluminescent biochip-based immunoassays were employed. Ligands were immobilized and stabilized to the biochip surface defining an array of twenty discrete test sites (15 Tier 1 assays and 5 Tier 2 assays). The assays were designed to detect both parent and urinary metabolites where applicable. The signal output is inversely proportional to the concentration of drug in the sample. Tier 1 assays included were: Amphetamine (AMPH), Methamphetamine (MAMP), Barbiturate (BARB), Benzodiazepine Class 1 (BENZ1), Benzodiazepine Class 2 (BENZ2), Cannabinoids (THC), Cocaine/Benzoylecgonine (BZG), Hydromorphone (OPDS), Meprobamate (MPB), Methadone (MDONE), Opiates (OPIAT), Oxycodone (OXYC1 and OXYC2), Phencyclidine (PCP), and Zolpidem (ZOL). Tier 2 assays included: Buprenorphine (BUP), Dextromethorphan (DMP), Fentanyl (FENT), Tramadol (TRM), and Tricyclic antidepressants (TCAs). Assays were applied to both the fully automated Evidence Analyser and the semi-automated Evidence Investigator, which have dedicated software to process, report and archive the data produced. The data presented correspond to the Evidence Investigator Analyser. The results are semi-quantitative and the sample volume required is 10µL of neat urine.

Results: The following limits of detection (LOD) for Tier 1 drugs were obtained: AMPH 51.2ng/mL, MAMP 12.6ng/mL, BARB 27.5ng/mL, BENZ1 0.4ng/mL, BENZ2 3.7ng/mL, THC 3.1ng/mL, BZG 4.8ng/mL, OPDS 7.2ng/mL, MPB 23.9ng/mL, MDONE 5.5ng/mL, OPIAT 11.4ng/mL, OXYC1 5.1ng/mL, OXYC2 0.3ng/mL, PCP 1.0ng/mL, ZOL 1.1ng/mL. Tier 2 drugs assays presented the following LODs: BUP 0.2ng/mL, DMP 0.8ng/mL, FENT 0.3ng/mL, TRM 0.7ng/mL, TCA 1.7ng/mL. The LODs stated were less than 50% of the cut-off for all the assays and the cut-offs were within the values stated in the recommendations. Inter-assay precision (n=15) was assessed for 3 replicates over 5 separate runs +/-50 % of the cut-off, and the CV (%), ranged between 6.9-19.8%. The recovery of spiked samples prepared +/- 50% of the cut-off value ranged from 78% to 127%.

Conclusion: The results indicate applicability of BAT to the simultaneous screening of drugs associated with DUID and included in Tier 1 and Tier 2 drugs under reported recommendations. The twenty immunoassays arrayed on the biochip surface presented lower LODs than the recommended cut-offs in urine. This methodology allows for multi-analytical screening of samples, leading to test consolidation and increased screening capacity in test settings.

Keywords: Drug Impaired Driving, Biochip Array Technology, Tier 1 Screening

P21

More Than Recovery – Cleanliness - A Thorough Approach to Oral Fluid LC/MS Analysis with OFC Devices

Laura Snow*, Shahana Huq, Sean Orlowicz, Seyed Sadjadi; Phenomenex, Inc., PhenoLogix Dept., Torrance, CA

Background/Introduction: Drug testing in oral fluid is of increasing interest due to the non-invasive nature and ease of sample collection. Many commercially available oral fluid collection devices (OFC Devices) provide stabilizing buffers to preserve analytes while the samples are in transit to the testing lab. Additives in the stabilizing buffers, while necessary for prolonging the longevity of the compounds, may impact some aspects of LC/MS analysis. In this work, we demonstrate a cleanup procedure utilizing ion-exchange solid phase extraction (SPE) methods on two popular OFC Devices that substantially reduces these preservative buffer additives while producing a good degree of analyte recovery and reproducibility.

Objective: Using SPE, we substantially reduced additives in oral fluid samples while producing good analyte recovery and reproducibility.

Methods: Neat preservative buffer and SPE-extracted oral fluid samples from OFC devices were qualitatively analyzed via LC/MS. Chromatographic separation was carried out on a Kinetex C18, 50x2.1mm, 2.6um column with mobile phases (MP) consisting of 0.1% formic acid in water and methanol. A Sciex 4000 QTRAP LC/MS/MS with ESI source was used for detection. Major regions of ion suppression were identified through post-column T-infusion of codeine. Samples were further characterized by collecting Q1 scans from 100 to 2000 Da under positive and negative polarity modes. Recovery and precision data were collected on a Sciex API 5000 LC/MS/MS system. A Kinetex Biphenyl 50x3.0mm, 2.6um column connected to an Agilent 1260 LC system was used with MP conditions similar to the qualitative experiments above. The probe compounds selected were pain panel drugs including opiates (morphine, 6-MAM, codeine), amphetamines (MDMA, MDEA, MDA), synthetic opiates (hydromorphone, hydrocodone), benzodiazepines (lorazepam, temazepam), COOH-THC, barbiturates (phenobarbital, pentobarbital), PCP and carisoprodol. Two MRM transitions for each analyte and one for each deuterated IntStd were monitored. Sample clean-up was performed using 30mg/3mL Strata-X-A (strong anion exchange) and Strata-X-C (strong cation exchange) sorbents. Collection pads, wetted with 1mL of human oral fluid (spiked with analytical standards) were inserted into transport tubes containing preservative buffer and allowed to stand overnight. 0.5mL of this combined mixture was diluted (1:2) with either a 1% HCOOH solution (basic compounds) or 1% NH_4OH solution (acidic compounds) prior to extraction. A variety of solvents in pure form and diluted with water were tested as SPE strong wash to ascertain the extent of additive loss/recovery from OFC device preservative buffer. For acidic compounds, the final elution reagent was composed of ACN:MeOH:HCOOH at a ratio of 47.5:47.5:5. Similarly, for basic compounds the elution reagent was made of ACN:MeOH:NH_4OH at a ratio of 47.5:47.5:5.

Result: A 50% aqueous acetone wash removed the majority of the additives from both device buffers. The majority of the 49 drugs tested were basic compounds and recoveries ranged from 46 to 80% for device 1 and 45 to 104% for device 2. Acidic analytes, barbiturates, COOH-THC and lorazepam recoveries ranged from 60 to 80% for device 1 and 63 to 93% for device 2. Neutral compounds meprobamate and carisoprodol were not well-retained on either SPE sorbent, for either device, and produced recoveries <16%. Precision was good with %CV <19% for all replicates (n=4).

Conclusion/Discussion: We were able to remove the majority of the additives from the OFC device buffers with the use of 50% aqueous acetone wash. This procedure yielded good recovery of most of the tested analytes. The neutral compound recovery was less than desirable.

Keywords: Oral Fluid, SPE, LC-MS/MS

P22

Prevalence of Hydrocodone and Oxycodone and their Metabolites in Oral Fluid of Pain Management Patients

Donna J. Coy*, Graham N. Keto, Piyadarsha Amaratunga, Bridget Lorenz Lemberg; Forensic Fluids Laboratories, Kalamazoo, MI

Background/Introduction: Oral Fluid (OF) is an emerging matrix for use in drug testing because collection is noninvasive and can be easily observed. Because OF is an ultra-filtrate of blood, it is ideal for identifying recent drug use, medication compliance, and has potential for use in therapeutic drug monitoring. Detection of metabolites in OF can further confirm proper use of medication, or possible diversion. Semi-synthetic opioids are widely prescribed for chronic pain and have a high potential for abuse. Interpretation of opioid test results can be complicated; as many opioids share metabolites and some metabolites are medications themselves. Two of the most commonly used opioid analgesics are hydrocodone (HC) and oxycodone (OC). Studies have shown the major metabolites of HC and OC in OF are norhydrocodone and noroxycodone, respectively. The metabolites hydromorphone (HM) from HC and oxymorphone (OM) from OC have also been found in OF, but often times below the lower limit of quantitation (LLOQ). As techniques become more sensitive, detection of these metabolites at low concentrations can enhance interpretation of test results.

Objective: The goal of this study was to determine the prevalence of metabolites for HC and OC in OF. Oral fluid samples positive for HC and OC were analyzed for presence of their metabolites: NHC, HM, NOC, and OM.

Methods: OF samples were collected using the Intercept® i2™ collection device. Samples were sealed and sent at ambient temperature to Forensic Fluids Laboratories. Samples were analyzed on the day they were received. Solid phase extraction was performed using Agilent PCX Plexa 96-well plate followed by LC/MS/MS analysis by Waters Acquity UPLC I-Class LC system paired with Waters Xevo TQ-S mass spectrometer. LLOQ was 0.3 ng/mL in neat OF for all analytes. All samples were tested for the presence of HC and OC and metabolites (NHC, HM, NOC, and OM). In addition, morphine (MOR), codeine (COD), and 6-acetylmorphine (6MAM) were tested.

Result: From February to April 2016, 1680 and 991 HC and OC positive samples were identified, respectively. The median concentration (range) of HC was 48.3 (0.6-941.5 ng/mL). Median concentrations (ranges) for NHC and HM were 3.3 (0.3-42.5 ng/mL) and 0.6 (0.3-7 ng/mL), respectively. At least one metabolite was present in 89.9% of samples and 22.7% had both metabolites present. Concentration of analytes were HC > NHC > HM. Twelve samples were found to have HM > NHC, with 9 having HM, MOR, or COD as a prescription. Oxycodone results showed a similar pattern. The median concentrations (ranges) of OC, NOC, and OM were 100.5 (0.5-3487 ng/mL), 23.1 (0.6- 398 ng/mL), and 1.2 (0.3- 30 ng/mL), respectively. At least one metabolite was present in 95% of samples and 60.8% had both metabolites present. Concentrations of analytes followed the same pattern as HC, OC > NOC > OM. Six samples had OM > NOC, with 3 having OM prescriptions or likely diversion.

Conclusion/Discussion: Previous studies focusing on HC and OC metabolites in OF have shown HM and OM are often found at low frequency, if at all. This can be contributed to their low concentrations in OF and limitations based on LLOQ. In this study we were able to find HM and OM at higher frequencies (23% and 62.2%, respectively) in OF samples positive for HC and OC with a LLOQ of 0.3 ng/mL. The results show the need for greater sensitivity when measuring these metabolites in OF, as 74% and 44% of HM and OM concentrations were below 1 ng/mL, respectively. Understanding the metabolic profile of HC and OC in OF can contribute to interpretation of medication compliance when confronted with multiple opioid prescriptions and potential diversion.

Keywords: Oral Fluid, Hydrocodone, Oxycodone, Pain Management

P23

Developing and Validating a Contemporary Scope for Synthetic Cannabinoid Testing in the 2016 NPS Drug Market

Barry K. Logan*[1,2], Sherri L. Kacinko[1], Joseph W. Homan[1]; [1]NMS Labs, Willow Grove, PA, [2]Center for Forensic Science Research and Education, Willow Grove, PA

Background/Introduction: Synthetic cannabinoids present a challenge to the forensic toxicology community. Since their first appearance in the United States in late 2009, labs have had to continually update their methods to detect the newest available compounds.

Objective: The objective of this presentation is to describe the importance of maintaining a current scope for testing biological samples for synthetic cannabinoids. Additionally current trends in synthetic cannabinoids will be discussed.

Methods: Positivity trends for casework submitted for synthetic cannabinoid analysis was evaluated along with information from drug chemistry casework, client requests and drug user forums to determine a scope for an updated panel. The proposed scope contained 47 analytes including 13 new analytes. A new two-dimensional hybrid triple quadrupole linear ion trap mass spectrometry (QTRAP, Sciex 5600) method was developed and validated due to its sensitivity and ease of updating the scope. After being fortified with internal standard, 0.5 mL of blood was buffered with 0.5 mL TRIS HCl (1.0 M, pH 10.2). Analytes were extracted using 3.0 mL of methyl tert-butyl ether. The organic layer was evaporated to dryness. Samples were reconstituted in 500 microliters of 50:50 Deionized water:Methanol with 0.1% formic acid and injected on the QTRAP. Chromatographic separation was achieved on a C18 column with gradient elution. Any sample with an area count ≥ 50% of the cutoff calibrator and a spectral library match score ≥ 60% proceeded to confirmation testing. The QTRAP method was validated by evaluating precision around the cutoff, sensitivity, specificity, interfering substances, and carryover. Precision was evaluated by analyzing 20 replicates each of controls fortified at 50% of the cutoff and 200% of the cutoff concentration. Performance was considered acceptable if 90% of the samples gave the expected results. Sensitivity and specificity were evaluated using 90 samples which had previously tested positive for at least one analyte and 150 additional spiked samples. Minimum sensitivity and specificity of 80% was required for method acceptance. Interferences were evaluated by analyzing 83 commonly encountered drugs including over-the-counter medications, prescriptions drugs and illicit substances to confirm no positive results were triggered. A blank sample injected after a sample fortified with a high concentration of analyte was used to evaluate carryover. Finally 10 samples which did had previously been analyzed and reported as "None Detected" were reanalyzed using the fully validated screen and confirmation procedures.

Result: From Mar 2015-March 2016 an average of 172 samples were analyzed/month; 30.2% of samples tested positive for at least 1 analyte. The positivity rates for the most prominent compounds are indicated below.

Drug	Positivity
ADB-CHMINACA	12.9%
AB-CHMINACA	14.1%
ADB-FUBINACA	1.5% - 3.8%*
XLR-11	4.6%

* Mar – Oct 2015 vs. Nov 2015 – Mar 2016 respectively

Validation was successful for 44 of the 47 analytes evaluated. JWH-081 and JWH-210 were removed from the updated scope due to low positivity and validation failure. EG-018, a new analyte, did not pass validation and thus was not included in the final scope. Validation was successful for all other compounds. Among 90 samples previously analyzed, on retest 19 screened positive by QTRAP for new analytes. Cases with sufficient sample were reanalyzed on the appropriate confirmation method. Results are shown below:

	# Screened positive	# Sent to confirmation	# Confirmed positive
PX3	4	4	4
EG-2201	5	2	0
FUB-AMB	1	1	0
MA-CHMINACA	3	3	3
NM-2201	6	6	6

Keywords: Synthetic Cannabinoids, QTRAP, Novel Psychoactive Substances

P24

A Retrospective Study of Heroin Findings in Hair Samples Over an 8 Year Period, 2008-2015

Neil Stowe*, Virginia Hill, Michael Schaffer: Psychemedics Corporation, Culver City, CA

Background/Introduction: The rise in heroin use has been documented, and is often considered as reaching epidemic levels, with an alarming increase of heroin related overdoses occurring within the years 2010-2014. The widespread increase in heroin use has been linked in the literature to the abuse of pain relieving opioids, with initial dependence beginning at the prescription opiate level and subsequently transitioning into heroin abuse due to heroin's comparatively low price, ease of use versus improved prescription opiate formulations designed to prevent abuse and that heroin is more readily obtainable than prescription opiates.

Objective: Herein, we present our findings of hair samples positive for the heroin metabolite 6-acetyl morphine (6AM) in the years 2008-2015 using samples that were analyzed by the Psychemedics Laboratory. Using 2008 as our start date for normalization, we provide the percentage of 6AM positive hair samples over the 2008-2015 time period. We also have tabulated the range of hair concentrations of 6AM and morphine from 2008-2015, as well as the ratio of 6AM to morphine over the same time period.

Methods: Samples were identified as presumptive positive by an immunoassay screen. Samples were confirmed using an extended washing procedure before LC/MS/MS quantitation. Cutoffs for the screening and confirmation were set at 2 ng/10 mg hair.

Result: When normalized to 2008, we have found that the percentage of 6AM positive hair samples has increased greater than two-fold over the 2008-2015 time period (**Table 1**), similar to recent morbidity reports by HHS. The tabulated hair concentration results are found in **Table 2**.

Table 1. Normalized 6AM Positive Rates, Beginning 2008.

Year	2008	2009	2010	2011	2012	2013	2014	2015
Normalized Result	1.0	1.05	0.91	1.00	1.04	1.25	1.83	2.40

Table 2. Tabulated Results for 2008-2015 6AM and Morphine Data.

Year	6AM Range (ng/10 mg)	Median 6AM (ng/10 mg)	Morphine Range (ng/10 mg)	Median Morphine (ng/10 mg)	Avg. Ratio 6AM:Morphine
2015	2.00 to 200.00	9.46	0.53 to 173.00	9.76	1.35 ± 1.24
2014	2.05 to 301.87	9.96	0.60 to 326.38	9.96	1.33 ± 1.10
2013	2.00 to 151.96	7.92	1.33 to 183.81	11.58	1.12 ± 0.98
2012	2.00 to 200.00	6.72	0.64 to 138.05	8.2	1.23 ± 1.08
2011	2.01 to 200.00	7.21	0.70 to 200.00	6.66	1.67 ± 1.51
2010	2.01 to 200.00	6.94	0.67 to 122.18	7.74	1.46 ± 1.39
2009	2.02 to 200.00	6.99	0.70 to 182.49	9.46	1.32 ± 1.17
2008	2.02 to 115.97	7.18	0.51 to 109.73	8.18	1.42 ± 1.31

Conclusion/Discussion: In line with recent reports, the 6AM positive rate has increased from 2008-2015.

Keywords: Hair, LC-MS/MS, Heroin

P25

eXtreme Filter Vial Extraction for the Detection of 11 Antidepressants in Oral Fluid Samples

Sarah Muller*[1], Jill Yeakel[1], Lisa Wanders[2]; [1]Lehigh Valley Toxicology, Bethlehem, PA, [2]Thomson Instrument Company, Oceanside, CA

Background/Introduction: Oral fluid has gained popularity in recent years as a biological specimen that can be utilized for drug testing. Oral fluid can detect recent drug use, allows for observed and easy collection and provides an almost adulteration proof sample for testing. Solid phase extraction has been customary as the reliable and sensitive method to extract compounds of interest from oral fluid. However, solid phase extraction can be time consuming and requires the use of extraction equipment such as vacuum or positive pressure manifolds and evaporators. A new extraction technique has been introduced onto the market that eliminates the need for specialized equipment, significantly reduces solvent waste, and requires much less time to process the samples while still producing sensitive and accurate results. Thomson eXtreme Filter Vials provide a simple and efficient extraction technique that has demonstrated adequate analyte recovery, reduced matrix interferences and the elimination of solvent waste and other consumables. This project specifically explores the efficacy of these vials in extracting a wide range of antidepressants in oral fluid specimens.

Objective: The objective of this research was to evaluate Thomson eXtreme Filter Vials for their efficiency and accuracy in extracting antidepressants from oral fluid.

Methods: Twenty-nine de-identified oral fluid samples were provided for the study along with a list of patient demographic information. The samples were collected using the OraSure Intercept I2he collection device. Approximately one milliliter of oral fluid is collected into two milliliters of Intercept i2hE Diluent. Oral fluid samples underwent a filter vial extraction prior to analysis on a liquid chromatograph tandem mass spectrometer (LC-MS/MS). The compounds included in the testing panel were opioids, benzodiazepines, drugs of abuse and antidepressants. Antidepressants analyzed included amitriptyline, sertraline, fluoxetine, trazodone, venlafaxine, citalopram, and select metabolites.

Results: An extraction technique was developed to rapidly filter oral fluid specimens prior to analysis on the LC-MS/MS. The method required 100 uL sample, 100 uL mobile phase, and 20 uL internal standard. The calibration range was established between 5 ng/mL to 200 ng/mL. Controls sufficiently passed quantitatively and qualitatively within established ranges of targeted values (15 and 150 ng/mL respectively). To obtain the undiluted concentration of analyte in the sample, values were multiplied by a factor of three. Adequate chromatographic separation of all tested analytes was achieved while still attaining optimal sensitivity. Of the samples analyzed, seven were positive for antidepressants (sertraline, venlafaxine, trazodone, fluoxetine and citalopram) and their metabolites. These results were consistent with the provided medication lists. Samples were also simultaneously analyzed for opioids, benzodiazepines, barbiturates and drugs of abuse.

Conclusion/Discussion: The developed method utilizing the eXtreme filter vials proved successful in extracting and detecting antidepressants and metabolites present in oral fluid with a high level of sensitivity and accuracy. A simple, rapid, and accurate comprehensive method was developed for the detection of 48 drugs in oral fluid samples.

Keywords: Filter Vials, Antidepressants, Analytical Toxicology

P26

Application of SWATH™ Acquisition for Broad Based Forensic Toxicology Drug Screening of Oral Fluid Using Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF)

Alex J. Krotulski*[1], Amanda L.A. Mohr[1], Melissa Friscia[1], and Barry K. Logan[1,2]; [1]The Center for Forensic Science Research and Education, Willow Grove, PA, [2]NMS Labs, Willow Grove, PA

Background/Introduction: In contrast to data-dependent acquisition methods, Sequential Windowed Acquisition for all Theoretical mass spectra (SWATH™ acquisition) (Sciex, Ontario, Canada) is a data-independent acquisition mode that allows for broad-based drug screening and the acquisition of accurate mass MS/MS data for all possible candidates. SWATH™ acquisition employs isolation windows designed to allow only specific precursor mass-to-charge ions in a given range through the quadrupole, allowing for more specific identification of fragments produced in the collision cell. This added level of specificity results in the ability to search the accurate mass MS/MS data acquired against an existing accurate mass library, including fragmentation data, providing additional confidence during data processing for candidate analyte identification.

Objective: This project used SWATH™ acquisition technology for the simultaneous detection of therapeutic and abused drugs during a broad-based drug screen.

Methods: Oral fluid (OF) samples were fortified with 259 therapeutic and abused drugs including many novel psychoactive substances (NPS), at sub-therapeutic, therapeutic/recreational, and toxic concentrations. OF samples (0.5 mL) were extracted using Borax buffer (0.1M, pH 10.4) and n-butyl chloride/ethyl acetate (70:30). Analysis was performed in triplicate over three days consistent with SWGTOX validation guidelines for qualitative analysis. Analysis was performed using a TripleTOF™ 5600^{+} mass spectrometer (Sciex, Ontario, Canada) coupled with a Shimadzu Nexera XR ultra high performance liquid chromatograph (Shimadzu, Kyoto, Japan). A reverse phase gradient was performed using ammonium formate (10mM, pH 3) and methanol/acetonitrile (50:50) for chromatographic separation on a Phenomenex® Kinetex C18 analytical column (50mm x 3.0mm, 2.6µm) at a flow rate of 0.4 mL min^{-1} for a total run time of 15.5 minutes. Precursor ions were acquired by TOF MS scan (100-510 m/z) via positive electrospray ionization. Precursor isolation was performed using SWATH™ acquisition, consisting of 27 overlapping isolation windows. Fragmentation was achieved using a rolling collision energy of 35±15eV. The acquisition total cycle time was 0.77 seconds. Data processing was performed using PeakView Software (Version 2.2, Sciex, Ontario, Canada) with an extracted ion chromatogram (XIC) list containing 1463 compounds, of which 382 had fragment and retention time data, and accurate mass library containing 1790 compounds. Criteria for positive analyte identification included the following: mass error within 10 ppm; isotope difference less than 50%; retention time within 0.35 minutes of the reference; and library score greater than 70. Additionally, a chromatographic event must have an intensity of 800 counts; a signal-to-noise ratio of 10; and acceptable peak shape.

Results: Positivity rates were calculated based on the number compounds correctly identified during analysis: mass error (100%), retention time (100%), isotope difference (98.5%), library score (95.4%), intensity (97.3%), and chromatography (98.8%). The calculated difference between theoretical and actual isotope abundance was found to be more variable than anticipated, and therefore the criteria was increased from 20 to 50%. Compounds not identified based on library score were attributed to low intensity ions and convoluted spectra. Opiate and opioid analysis by TOF MS resulted in poor chromatography; but with the use of SWATH™ and fragment XICs, all opiates and opioids were able to be positively identified, with the exception of oxymorphone due to low intensity. OF samples collected at an electronic dance music (EDM) festival were analyzed using this method and processed for the presence of NPS and other drugs of abuse.

Conclusion/Discussion: The application of SWATH™ acquisition for broad-based drug screening was successful for the analysis of 259 therapeutic and abused drugs fortified in OF. Retention time data, accurate fragment mass data, and accurate mass library spectra were acquired and used during data processing. Mass error, isotope difference, retention time difference, and library score criteria were established for processing of SWATH™ acquisition data.

Keywords: SWATH™, QTOF, Oral Fluid

P27
Analysis of Potency, Pesticides and Mycotoxins in Marijuana

Xiaoyan Wang, Brian Kinsella, Tina Fanning, **Danielle Mackowsky*,** Michael Telepchak; UCT, LLC, Bristol, PA

Background/Introduction: An increasing number of jurisdictions within the United States have legalized the use of medicinal marijuana. Consistent regulations regarding acceptable limits of pesticides and mycotoxins, as well as uniform testing methods for potency are lacking. A study was conducted using a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction with UCT's newest purification product, SpinFiltr^{TM}, for the analysis of four cannabinoids ,48 pesticides and four mycotoxins in marijuana. In addition, the analysis of seized marijuana samples for potency and pesticides is also discussed.

Objective: To develop an extraction method for five mycotoxins, four cannabinoids(THC, THC-A, THC-COOH,THC-OH) and 48 pesticides in marijuana.

Method: Marijuana samples were ground into a fine powder using a SPEX 6770 freezer mill. 1g of the marijuana powder was then added to a 50-mL centrifuge tube containing internal standard and 10 mL of water. Samples were then vortexed, followed by hydrated for 15 minutes at 60 °C. 10 mL of acetonitrile with 2% formic acid was then added to the centrifuge tube along with QuEChERS(Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction salts from pouches (**ECMSSC-MP**). Salt agglomerates were broken up by votexing the tubes for 10 seconds. The tubes were then shaken for 1 minute at 1000 stroke/min using a SPEX Geno/Grinder. Samples were then placed in a centrifuge and spun for 5 minutes at 3000 rcf, resulting in three distinct layers.

Pesticide and mycotoxin analysis was performed by transferring 1 mL of the supernatants to UCT SpinFiltr^{TM} cleanup tubes (**ECQUSF54CT**). The tubes were vortexed and then spun down for 2 minutes at 3000 rcf. The extract was added to 2-mL auto-sampler vials for analysis.
Additional supernantant was used for cannabinoid analysis, which involved serial dilutions of the QuEChERS extracts. The diluted samples were spiked at 50 and 150% of the target concentration so that standard addition calculations could be performed.
Samples were then anaylzed by LC/MS/MS (Thermo Scientific UltiMate 3000 LC system coupled to TSQ Vantage tandem MS) equipped with an UCT Aqueous C18 HPLC column.

Result: Due to the various regulations between states, a wide panel of commonly encountered pesticides was selected for this study, in addition to the four mycotoxins selected. Quantitation was performed against a 6-point matrix-matched calibration curve prepared in unspiked marijuana extract. For most compounds the absolute recovery was in the range of 70-100% and the reproducibility was <10%. Seized street grade marijuana was found to have a variety of pesticides at concentrations higher than what was observed in the medical grade product.

Both medical and recreational samples had a percentage of THC per sample that ranged from 0.9-1.7. In addition, medical marijuana samples were specifically tested for tertrahydrocannabinolic acid (THCA-A), due to its potential therapeutic properties. On average, 17% of the total weight in each medical marijuana sample came from the presence of THCA-A.

Conclusion/Discussion: A fast and effective method was developed for the determination of 48 pesticide residues, four mycotoxins and cannabis potency in seized recreational and medical marijuana samples. All analytes of interest were extracted using the QuEChERS approach, followed by either an additional cleanup using a UCT SpinFiltr^{TM} for pesticide and mycotoxin analysis, or serial dilutions for cannabinoid potency testing. Analysis of the samples was performed by LC-MS/MS utilizing a Selectra® Aqueous C18 HPLC column which allowed for improved retention of the more polar pesticides included in the method. With the widespread legalization of marijuana, this simple method will be beneficial for implementing regulatory testing and for the analysis of additional compounds of interest beyond this application.

Keywords: Marijuana, QuEChERS, Mycotoxins

P28
Quantitative Measurement of Carbamazepine in Oral Fluid by LC-MS-MS

Piyadarsha Amaratunga*, Bridget Lorenz Lemberg, Dave Lemberg, Pankaj Lamba, Nabila Farooq, Varma Penumetch, Graham Keto; Forensic Fluids Laboratories, Kalamazoo, MI

Background/Introduction: Measuring antiepileptic drugs in body fluids to aid drug therapy is an established practice in the treatment of epilepsy. Carbamazepine, sold under the trade name Tegretol, is a medication used to treat epilepsy. Carbamazepine is an anticonvulsant that works by decreasing nerve impulses that cause seizures and pain. Carbamazepine comes as a tablet, a capsule and a liquid suspension and it is taken two or four times per day. The goal of therapeutic drug monitoring is to improve the clinical outcome of the treatment by measuring drug concentration levels. Measuring serum levels of drugs has conventionally been the practice to evaluate the bioavailability of antiepileptic drugs. There has been extensive interest in the use of oral fluid as an alternative matrix for therapeutic drug monitoring. The advantages of oral fluid include noninvasive sample collection, observed collection process and its ability to reflect drug concentrations equivalent to that of serum. Ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS-MS) is the current method of choice for confirmation of drugs in biological matrices. UHPLC-MS-MS requires minimum sample preparation and it can detect a large number of compounds within a short period with high resolution and sensitivity. Oral fluid testing methods of carbamazepine are important in a clinical laboratory. In a production setting, the methods should clean up the samples and quantify the analyte with minimal sample preparation in order to fit into a high-throughput production environment.

Objective: To develop a rapid and sensitive UHPLC-MS-MS method for quantitative measurement of carbamazepine in oral fluid.

Method: Oral fluid samples were collected with a Quantisal™ collection device. The Quantisal™ collection device consisted of a cellulose pad, an extraction buffer, and a plastic tube. There were 3 mL of buffer in the collection device. The cellulose pad absorbed 1 mL of oral fluid during the collection process. Because the original oral fluid was diluted with the extraction buffer during the extraction, detected drug concentrations were adjusted accordingly. The collected oral fluid samples were purified with solid phase extraction (SPE) prior to LC-MS-MS analysis. SPE was performed in a vacuum manifold using an Oasis MCX cartridge. Oasis MCX cartridge was selected because it is a cation exchange sorbent that selectively separates and concentrates basic drugs during the extraction. SPE process is as follows: 400 µL sample and 0.75% phosphoric acid in water were mixed in a rotary shaker. The wells were preconditioned with methanol and the samples were loaded into the wells. The wells were washed with 0.75% phosphoric acid in water and methanol. The compounds were eluted with 4% NH_3 in ethyl acetate: methanol (75 : 25, v/v). The eluent was dried under nitrogen and reconstituted with mobile phase A. Stability of carbamazepine in the collection device wasn't evaluated during the study.

Chromatographic separation was performed on an Acquity UHPLC system (Waters) equipped with Zorbax SB-C8 RRHD (100X2.1 mm, 1.8 mm) analytical column (Agilent Technologies). Electrospray ionization mass spectrometry was performed on a TQD instrument (Waters). Analysis was performed in positive ionization (ESI+) and multiple reaction monitoring (MRM) mode. MRM transitions for carbamazepine was 237>194 m/z and 237>179 m/z.

Results/Conclusions: The developed method was validated according to Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines. The linear dynamic range was 10-1000 ng/mL with a lowest limit of quantitation (LLOQ) of 10.0 ng/mL for carbamazepine. The acceptance criterion for the accuracy was that % relative error should be ≤20% for QC samples. The acceptance criterion for the precision was that the %CV should be ≤20% for QC samples. The results of the accuracy and the precision values were within the acceptance criteria for carbamazepine. In addition, selectivity, matrix effect and recovery were calculated for the LC-MS-MS method. Carbamazepine D10 was used as the internal standard. Thirteen authentic samples were tested with the current method to evaluate the applicability of the method.

Keywords: Carbamazepine, LC-MS-MS, Oral Fluid

P29

Detection of Cocaine and its Major Metabolites in Rodent Bone Following Outdoor Decomposition After Chronic Cocaine Administration Using 2D-LC/MS/MS

Malorie Mella*[1,2], Sabra Botch-Jones[1], Claude Mallet[2], Tara Moore[1]; [1]Boston University School of Medicine, Boston, MA, [2]Waters Corporation, Milford, MA

Background/Introduction: In the field of forensic toxicology, several challenges exist with quantification analysis of cocaine and metabolites in post mortem samples. Cocaine can prove difficult to detect and quantify in blood, urine, and soft tissues following extensive decomposition. Alternative matrices, such as hair, nails, and bone could prove useful in detecting chronic drug use in post-mortem toxicology cases. Detection and quantification of drugs in complex matrices is difficult to accomplish due to time-consuming extraction processes, and inability to detect an analyte at trace levels. Further, analysis of drugs in hard tissues, such as hair and bone, has only been attempted in recent years. Even fewer studies have investigated for the detection of drugs following decomposition of remains, specifically outdoor decomposition.

Objective: The objective of this study was to develop a robust extraction and clean up methodology, in which a homogenization step precedes, to efficiently extract drugs from complex matrices, reach a target limit of detection (LOD) and to maintain instrument performance.

Method: All rat specimens used for this study underwent 10-12 weeks chronic intravenous self-administration of cocaine. This was followed by a six-week period of abstinence, followed again by a three-week period of cocaine self-administration before being euthanized. Average daily dosages for each rat fell within a range of 13-19 mg/kg. 14 cocaine positive rats were placed outside and above ground in a gated facility for a period of 12 months. All recoverable pelt and skeletal samples were collected for testing. A second group consisting of 16 cocaine-positive rats was placed outside and above ground in a gated facility for 1 week. A group of 4 cocaine positive rats were removed for testing on the second week, and every week following. All recoverable skeletal samples were collected for testing. Drug free control rat bones were also acquired by placing drug-free rats outdoors, above ground, until full decomposition occurred. In this study, a method analyzing cocaine and its major metabolites benzoylecgonine and ecgonine methyl ester was developed.

After homogenization of whole bones, the extraction process was performed using a mixed mode reversed-phase/ion exchange sorbent, which yields two eluting fractions—one with neutral and acidic entities, one with basic analytes. The use of a 2D LC/MS/MS technology eliminates the need for a lengthy evaporation step in the extraction method. The chosen 2D LC/MS/MS used in this application was identified using a 6x6 automated method development protocol. The manual extraction of the bone samples was completed in less than an hour. The analysis was performed using 100µL of the final organic solvent (MeOH) extracts.

Result: The limit of quantitation (LOQ) for cocaine and its metabolites was measured at 100ng/g sample material. The response factor of analytes was high enough that the limit of detection (LOD) was estimated at 10ng/g (10ppt).

Conclusion/Discussion: The micro extraction protocol combined with a multi-dimension chromatography used in this study decreased sample preparation time without sacrificing the quality seen with current single dimension chromatography techniques. The procedure developed in this study can be utilized on bone and completed in less than an hour before injection of 100 L final extract into the 2D-LC/MS/MS system.

Keywords: LC/MS/MS, Multidimensional Chromatography, Bone

P30

Knowing Your B's and C's: The Identification of 25B-NBOMe and 25C-NBOMe Biomarkers

Renata Nascimento*[1], Justin L. Poklis[2], Joseph K. Ritter[2], Carl E. Wolf[1,3], Alphonse Poklis[1,2,3]; Departments of [1]Forensic Science, [2]Pharmacology & Toxicology, and [3]Pathology at Virginia Commonwealth University, Richmond, VA

Background/Introduction: N-methoxybenzylmethoxyphenylethylamine (NBOMe) derivatives are a relatively new class of synthetic hallucinogens that were readily available on the internet before being placed into a Schedule I classification. These derivatives were first synthesized in 2000 and are potent serotonin 2A (5-HT2A) receptor agonists. Currently, NBOMe derivatives are sold as powders or on blotter paper with 25B-NBOMe (4-bromo-2,5-dimethoxyN-(2-methoxybenzyl)-phenylethylamine) and 25C-NBOMe (4-chlorine-2,5-dimethoxyN-(2-methoxybenzyl)-phenylethylamine) being two of the most commonly reported NBOMe analogues. Clinical presentations of severe NBOMe intoxication include tachycardia, agitation, hypertension, aggressive/violent behavior, excited delirium, hallucinations and continuous agitation and seizures which can persist for as long as three days. The identification and analysis of designer drug metabolites is essential to the assessment of drug exposure, and since these metabolites are generally formed *in vivo* and may not be available in the illicit drug market, their presence in biological matrices confirms exposure to the parent drug, serving as good biomarkers for drug identification.

Objective: To identify suitable metabolite biomarkers for the 25B-NBOMe and 25C-NBOMe analogues. If these metabolites are present in blood, urine or other specimen in greater abundance and/or have longer half-lives than the respective parents, they may be valuable biomarkers in postmortem toxicology, driving while impaired testing and all areas of clinical and forensic urine drug testing.

Method: Reference materials of 25B-NBOMe and 25C-NBOMe were obtained from Cerilliant Corporation. The analogues were incubated in freshly prepared mouse liver microsomes. These *in vitro* samples were used to generate phase I and phase II metabolites. This animal study was approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The 25B- and 25C-NBOMe metabolites formed by the microsomes were identified using both a Shimadzu GC-MS QP-2010 and a Waters Acquity Xevo TQD LC-MS/MS system.

Result: Seven phase I and six phase II metabolites were identified for both 25B-NBOMe and 25C-NBOMe from the mouse microsomal preparations. The O-demethylated metabolites, in which the demethylation occurred at either the ortho or meta position were found to be the most abundant metabolites. These results are consistent with previous studies results in our laboratory from the metabolism of 25I-NBOMe.

Conclusion/Discussion: The O-desmethyl metabolites were identified as the best urinary biomarkers for the determination of exposure to 25B-NBOMe and 25C-NBOMe. The identification of these metabolite biomarkers will be useful in the detection of NBOMe derivatives in clinical and forensic specimens, potentially reducing the occurrence of false negative results due to absence of parent drugs.

Keywords: 25B-NBOMe, 25C-NBOMe, Metabolite Biomarkers

P31

Sensitive, Easy and Economical Method for Analysis of THC and THC-COOH in Oral Fluid Using Novel Solid Phase Extraction Technology for Sample Preparation

Xiaolei Xie, Thomas Carrell, Marta Kozak, **Kristine Van Natta***; Thermo Fisher Scientific, San Jose, CA

Background/Introduction: Oral fluid testing for THC provides a convenient means of detecting recent cannabis use. Additional testing for a THC metabolite (THC-COOH) eliminates risk of positive results due to passive exposure and extends drug detection time. However, concentrations of the metabolite are typically in low pg/mL range and require sensitive analytical methods**.**

Objective: Develop a sensitive, easy and economical LC-MS method for analysis of THC and THC-COOH in oral fluid using novel fritless, low-elution volume solid phase extraction (SPE) plates for sample preparation.

Method: Oral fluid samples (diluted with preservation buffer) were spiked with internal standards (deuterated analogs) and mixed 1:1 with protein precipitation solution (acetonitrile). The samples were processed by SPE using SOLAμ SAX 96-well plates. The SPE method included conditioning, extraction, wash (50% acetonitrile) and elution (60 μL of 0.1% formic acid in acetonitrile) steps. Evaporation and reconstitution steps were not needed because of the low elution volume. A 50-μL aliquot of eluent was analyzed by liquid chromatography combined with triple quadrupole mass spectrometry in a 5-minute analytical run. Two SRM transitions were collected for each analyte to calculate ion ratio for confirmation. **C**alibration standards in synthetic oral fluid (THC-COOH range 5-1000 pg/mL, THC range 0.5-100 ng/mL) and QC samples (THC-COOH: 25, 100, and 500 pg/mL, THC: 2.5, 10, 50 ng/mL) were prepared in-house. Method precision, limits of quantitation (LOQ) and linearity ranges were determined by processing and analyzing 5 replicates of each calibration standard and each QC sample in 3 different analytical runs. Matrix effects were evaluated by spiking water and oral fluid samples from 5 donors at concentrations of 50 pg/mL and 5 ng/ml for THC-COOH and THC respectively and comparing chromatographic peak areas in oral fluid and water spiked samples. SPE extraction efficacy was also obtained using oral fluid from 5 donors.

Result: Lower limits of quantitation (defined as the lowest concentrations that had back-calculated values within 20% of nominal, RSD for 5 replicates within 20%, and ion ratio within required range) were 10 pg/mL for THC-COOH and 0.5 ng/mL for THC. The upper calibration range (equal to highest evaluated concentration) was 1000 pg/mL for THC-COOH and 100 ng/mL for THC. Within-batch precision was better than 9.5% and 3.0% for THC-COOH and THC, respectively. Between-batch precision was better than 8.4% and 3.2% for THC-COOH and THC, respectively. Limited matrix effects were observed and were corrected by internal standards. Absolute peak area recovery in spiked donor oral fluid samples compared to sample prepared in water were in ranges of 79.6-125% and 94.9-99.0% for THC-COOH and THC respectively. Carryover was not observed even at the highest calibrator concentration. SPE extraction efficacies were in ranges of 85.4-106% and 55.8-65.1% for THC-COOH and THC respectively.

Conclusion/Discussion: We developed a sensitive and easy method for analysis of THC and metabolite in oral fluid using novel SPE technology for sample preparation. This technology eliminates the evaporation and reconstitution steps, making sample preparation simple, fast and economical.

Keywords: Mass Spectrometry, Oral Fluid, THC

P32

Comparison of Two High-Resolution Mass Spectrometry Data Acquisition Methods for Screening, Quantitation and Confirmation of Compounds in Post-Mortem Blood

Kristine Van Natta*, Marta Kozak; Thermo Scientific, San Jose, CA

Background/Introduction: Forensic toxicologists need to quantitate a known set of compounds and screen for many more in as little time as possible. In the past, samples were screened either by GC-MS or immunoassay, both of which have significant limitations. GC-MS requires labor-intensive sample preparation including derivatization. Multiple immunoassays must be used to cover different compound classes, and immunoassays are not specific to a particular compound. LC-MS techniques allow for simpler sample preparation and identify individual compounds, not just a class.

Objective: Analyze post-mortem blood samples by LC-MS to correctly identify, quantify and confirm compounds of interest. Compare two mass spectrometric data acquisition methods for suitability.

Method: A single point calibrator (1-1000ng/mL, compound dependent), two QCs (one at half and one at double the calibrator concentration), and five unknown donor samples were processed by a collaborating laboratory using protein precipitation with a solution containing inter standards, evaporation and reconstitution with phosphate buffer. The calibrator and QCs contained 21 compounds selected to evaluate method performance, representing multiple drug classes routinely screened in forensic laboratories. Processed samples were subject to reversed phase chromatographic separation followed by detection on a hybrid quadrupole-Orbitrap™ mass spectrometer. Data was collected in two ways. In the first, the mass spectrometer collected high-resolution full-scan spectra at a resolution of 70k (FWHM at 200 *m/z*) along with data-dependent fragmentation spectra (FS-ddMS2) for any masses detected from a target list of over 400 compounds. In the second, full-scan spectra were again collected, followed by all-ion fragmentation (FS-AIF). Targeted compounds were identified using retention times and accurate mass *m/z* within 5 ppm mass accuracy from the full-scan data. Confirmation was accomplished either by matching the MS2 spectra to a spectral library or by presence of known fragments in the AIF data. Detection limits were evaluated using the 21 representative compounds in the calibrator and QCs. Quantitation was performed on the full-scan extracted ion chromatographic peak using the single point calibrator and linear-through-zero calibration curves. Identification accuracy was determined by analyzing unknown blood samples previously analyzed by the collaborating laboratory and comparing our results to theirs.

Result: All 21 of the known compounds in the calibrator and QC samples were detected and quantified. All QC compounds that had deuterated analogs as internal standards were within 20% of nominal concentration. Accuracies for some of the compounds that did not have deuterated analogs were outside of the 20% range, suggesting that analogs are needed if rigorous quantitation is required. For screening of the five unknown samples, ddMS2 and AIF performed equally well for confirmation of compounds within the concentration range of the QCs. The ddMS2 data still offers the strongest identification since the fragmentation spectra ”fingerprint” is collected for a specific precursor. AIF data is less specific since the fragments are generated by all ions eluting at the same time. The advantage of collecting AIF data is the ability to conduct confident retrospective data analysis using fragmentation data.

Conclusion/Discussion: The developed methods were able to both quantitate a known set of compounds and detect unknown compounds in post-mortem blood samples. Both methods performed similarly and met common industry requirements for sensitivity. Compounds from many classes were successfully and specifically screened in a single analytical run.

Keywords: Mass Spectrometry, Blood, Drugs of Abuse

P33

Screening, Confirmation and Quantitation of Synthetic Cathinones and Cannabinoids in Urine by High-Resolution Accurate-Mass Mass Spectrometry

Kristine Van Natta*, Marta Kozak; Thermo Scientific, San Jose, CA

Background/Introduction: Forensic laboratories need reliable and flexible methods for detecting novel psychoactive compounds such as synthetic cathinones and cannabinoids. The methods need to be easily modifiable to include new compounds. LC-MS is ideally suited for this type of application since it can easily detect different classes of compounds in a single analytical run.

Objective: To demonstrate the performance of high-resolution mass spectrometry for identification, confirmation and quantitation of synthetic cathinones and cannabinoids in urine.

Method: A single point calibrator at cutoff concentration (25-500 ng/mL, compound dependent) and two quality controls (QC) one each at 50% and 150% of the calibrator concentration were prepared by fortifying blank urine with 32 synthetic cathinones and cannabinoids. The calibrator, QCs and an unknown sample were processed by protein precipitation followed by dilution. Processed samples were subject to HPLC separation followed by detection on a hybrid quadrupole-Orbitrap™ mass spectrometer. Two chromatographic gradients were used. The first was a "fast and dirty" two-minute screening method that provided limited chromatographic separation of isobaric compounds. The second was a nine-minute gradient used for confirmation. The mass spectrometer collected high-resolution full-scan (FS) spectra at a resolution of 70k (FWHM at 200 m/z) along with data-dependent fragmentation spectra (ddMS2) for masses on the target list. The method could be easily modified by simply adding new masses to the target list. Compounds were identified using retention time and accurate *m/z* (5 ppm mass accuracy) from the full-scan data. Semi-quantitation was performed on the FS extracted ion chromatographic peak using the single point calibrator and linear-through-zero calibration curves. Confirmation was accomplished by spectral library matching with the MS2 spectra in both methods. Isotopic pattern matching was added to the longer method. To assess method performance, the calibrator and each QC sample, were injected ten times with each method to determine mass accuracy, peak area precision and quantitative performance. The unknown sample previously analyzed by collaborating laboratory was injected three times with each method to determine identification accuracy.

Result: Data from the short screening method showed mass accuracies within 1 ppm for all, except one compound, which was within 2.2 ppm. The long method, which was run several days after the short method and near the end of the recommended instrument calibration stability, showed mass accuracies within 3 ppm except for the same single compound, which was within 4.2 ppm. Peak area precision was better than 13.9% and 8.1% for all compounds and all concentrations for the short and long methods, respectively. Calculated concentration precision was better than 9.8 % and 8.5% across all compounds and all concentrations for the short and long methods, respectively. Three compounds were identified and confirmed in the unknown sample. The compounds were identified by retention time and accurate *m/z* from the FS data. They were confirmed with isotopic pattern matching and fragmentation spectra matching to a spectral library. A fourth compound was identified by *m/z*, retention time, and isotopic pattern matching. However, it failed the spectral matching. It was suspected that this compound might be a metabolite of one of the confirmed compounds. A literature search revealed a possible match which was confirmed with a theoretical fragmentation spectra match.

Conclusion/Discussion: The developed methods accomplished their goals of identifying, confirming and quantifying 32 synthetic cathinones and cannabinoids in urine. The short method was intended as a screening-only method, not requiring definitive confirmation. It surpassed that goal by also providing confirming fragmentation spectral matches. The longer confirmatory method provided better confirmation with higher quantitative precision and library matching scores.

Keywords: Mass Spectrometry, Novel Psychoactive Substances (NPS), Confirmation

P34

Characterization of 4-Fluoro-α-PHP and Pentylone in a Decedent

Kevin J. Schneider, **Tiffany M. Leusby***, Carol L. O'Neal; Virginia Department of Forensic Science, Northern Laboratory, Manassas, VA

Background/Introduction: The Northern District branch of the Office of the Chief Medical Examiner of Virginia submitted inferior vena cava blood and vitreous humor to the Department of Forensic Science for toxicological testing for a pending death investigation. The decedent's pathology revealed gunshot wounds, while evidence found at the scene suggested drug distribution activities. During the course of toxicological investigation, an alkaline drug screen of the blood showed a compound with mass spectral fragmentation consistent with a cathinone in addition to pentylone. An investigation was initiated to determine the identity of the unknown compound as well as confirmation of the pentylone in the blood.

Objective: The objective of this case study was the determination and quantification of an unidentified cathinone and pentylone in the blood of a decedent in order to assist the Medical Examiner. The complete toxicological investigation for this case will be of interest to toxicologists that may encounter these drugs in their work.

Method: Cathinone quantification was performed using an alkaline extraction from whole blood with added internal standard (methapyrilene). Briefly, blood samples were buffered using saturated borate buffer and extracted using THIA (78:20:2 v/v/v toluene:hexane:isoamyl alcohol). Organic extracts were back-extracted using 0.5 N H_2SO_4 and the organic phase was discarded. The solution was neutralized and alkalinized using 3:2 w/w $NaHCO_3$:K_2CO_3 and extracted using THIA. Final extracts were analyzed via GC-MS for spectral confirmation and GC-NPD for quantification (calibration range 0.010 – 1.0 mg/L). Values obtained using this method were semiquantitative because the method has not been fully validated for cathinones according to SWGTOX guidelines.

Result: Mass spectroscopic analysis of the uncharacterized compound determined the structure to be 4-fluoro-α-pyrrolidinohexiophenone (PHP). Designation was confirmed via retention time and mass spectral concordance with a purchased reference material. Semiquantitative analysis of the specimen for 4-fluoro-α-PHP and pentylone revealed concentrations of approximately 0.066 and 0.025 mg/L in whole blood, respectively. Additional findings in the blood included benzoylecgonine at 0.45 mg/L and oxycodone at 0.016 mg/L.

Conclusion/Discussion: This presentation details the first appearance of novel cathinone 4-fluoro-α-PHP in a case report or in the literature. While the authors were only able to generate semiquantitative values for 4-fluoro-α-PHP and pentylone (as no fully validated method currently exists), these give toxicologists an idea of approximate pharmacologically relevant concentrations of these two cathinones. The detailed concentrations are of additional value since the decedent expired due to physical trauma, meaning the concentrations are representative of recreational use and not an overdose situation.

Keywords: 4-Fluoro-α-PHP, Pentylone, Cathinone

P35
Despropionylfentanyl (4-ANPP) Emergence in Northern Virginia

Kevin J. Schneider*, Tiffany M. Leusby, Lucy Sale, Carol L. O'Neal; Virginia Department of Forensic Science, Northern Laboratory, Manassas, VA

Background/Introduction: During the course of standard toxicological examinations at the Virginia Department of Forensic Science Northern Laboratory, a series of cases screening positive for fentanyl via ELISA were determined to be negative for fentanyl and the fentanyl analogs acetylfentanyl and butyrylfentanyl. Further examination of these cases determined the presence of despropionylfentanyl (i.e., 4-anilino-N-phenethylpiperidine, 4-ANPP). Despropionylfentanyl has been described in the literature as a minor metabolite of fentanyl or a precursor sometimes used in the synthesis of fentanyl. These reports typically characterize despropionylfentanyl as pharmacologically inactive. However, recreational users have posted on drug forums that despropionylfentanyl is active and has an opioid potency of ~30× that of morphine (fentanyl is ~100× morphine potency).

Objective: This presentation describes an investigation initiated to better understand and characterize the emergence of despropionylfentanyl in toxicology specimens in the Northern Virginia area. An analysis of cases involving despropionylfentanyl along with additional drugs associated with these cases is presented here to assist in the interpretation of toxicology cases involving this potential drug of abuse.

Method: Fentanyl and fentanyl analog quantification was performed using an alkaline extraction from specimens with added internal standard (fentanyl-D5). Briefly, samples were buffered using saturated borate buffer and extracted using THIA (78:20:2 v/v/v toluene:hexane:isoamyl alcohol). Organic extracts were back-extracted using 0.5 N H_2SO_4 and the organic phase was discarded. The solution was neutralized and alkalinized using 3:2 w/w $NaHCO_3$:K_2CO_3 and extracted using THIA. Final extracts were analyzed via GC-MS using single ion monitoring (SIM). The calibration range was 0.0010 – 0.10 mg/L for fentanyl and 0.010 – 1.0 mg/L for despropionylfentanyl. Acetylfentanyl and butyrylfentanyl were determined qualitatively. Numerical values obtained for despropionylfentanyl using this method were semiquantitative because the method was not fully validated for despropionylfentanyl according to SWGTOX guidelines.

Result: Thirteen of the cases analyzed were found to contain despropionylfentanyl. Of the 13 cases, 4 were DUID, one was an overdose that did not result in death, and 8 were deaths associated with drug overdose. For DUID cases, approximate concentrations were 0.010 and 0.011 mg/L with the remaining two cases having despropionylfentanyl present at less than the lower limit of quantification for the method (LLOQ; 0.010 mg/L). The non-lethal overdose case also had despropionylfentanyl present at less than the LLOQ. For the overdose deaths, the approximate concentrations ranged from 0.012-0.073 mg/L (mean 0.049 mg/L, median 0.054 mg/L).

Conclusion/Discussion: Despropionylfentanyl has emerged in Northern Virginia casework as a noteworthy, potentially abused substance. Case analysis indicates that despropionylfentanyl is present at concentrations far in excess of those typically seen for fentanyl in casework. When considered with the data that show all but one case being negative for fentanyl and those cases with elevated fentanyl concentrations (e.g., >0.20 mg/L fentanyl) maintaining despropionylfentanyl concentrations below the limit of detection for this assay, it is clear that these despropionylfentanyl cases are not the result of fentanyl metabolism. Likewise, with no fentanyl present in most specimens, it is unlikely that these cases represent incomplete synthesis reactions.

All but one case testing positive for despropionylfentanyl contained either 6-acetylmorphine, morphine, or had elevated opiate ELISA screens, suggesting the potential for a batch of heroin that was adulterated with despropionylfentanyl. It is noteworthy that the case that screened negative for opiates contained fentanyl in combination with despropionylfentanyl, suggesting that the despropionylfentanyl present in this case could be the result of incomplete fentanyl synthesis. With heroin and adulterated heroin continuing to result in numerous overdoses and deaths in the Northern Virginia area, the Virginia Department of Forensic Science will continue to monitor this trend in hopes of better understanding new and emerging complexities associated with this epidemic.

Keywords: Despropionylfentanyl, 4-ANPP, Heroin

P36

Physiological Indicators and Driving Behavior Observed in Suspected Impaired Driving Cases Positive for 5F-ADB

Amanda Chandler*, Lyndsey Knoy, Brian Capron, Brianna Peterson; Washington State Patrol, Forensic Services Laboratory Bureau, Toxicology Laboratory Division, Seattle, WA

Background/Introduction: 5F-ADB (Methyl (S)-2[1-(5-fluoropentyl)-1H-indazole-3-carboxido]-3,3-dimethylbutanoate) is a synthetic cannabinoid that recently emerged in laboratory analyses.

Objective: This study reviews 38 case reports of suspected impaired driving cases that were positive for 5F-ADB.

Method: All cases were submitted to the Washington State Toxicology Laboratory from either Washington State or State of Alaska law enforcement agencies. Testing for synthetic cannabinoids was performed by AIT laboratories between November 2015 and May 2016.

Result: The concentrations of 5F-ADB in blood ranged from 0.2-1.2 ng/mL (mean - 0.4 ng/mL). Only 5 cases had other drugs detected; 3 had other synthetic cannabinoids and 2 had low levels of of carboxy-THC (<10 ng/mL). The driving population was 81% male with ages ranging from 21-54 years (mean age – 32 years). A review of the case histories submitted by the law enforcement agencies and/or Drug Recognition Expert Face Sheets demonstrated poor driving behavior; 50% were found passed out at the wheel and 32% were involved in collisions. Most of the subjects had bloodshot and watery eyes, slurred/slow speech, lethargy, and poor coordination. Of the 16 drivers that performed the standardized field sobriety tests, 9 cases had ≥2 clues for horizontal gaze nystagmus, 15 cases had ≥2 clues on the walk and turn test, and 11 cases had ≥2 clues for the one leg stand. Other observed effects include vomiting, seizures, and high blood pressure (>140/90 mm Hg).

Conclusion/Discussion: 5F-ADB is a potent new drug that is capable of causing significant impairment to driving.

Keywords: 5F-ADB, Synthetic Cannabinoids, Driving Impairment

P37
First Reported Postmortem Cases Involving Acetyl Fentanyl in Georgia

Thao P. Dang*, Brittany S. Gresham; Georgia Bureau of Investigation, Division of Forensic Sciences, Decatur, GA

Background/Introduction: Acetyl fentanyl is a schedule I synthetic opioid, also known as desmethyl fentanyl. It is an analog of fentanyl (schedule II). It can be an impurity in the production of fentanyl; however, it has no approved medicinal applications itself. In recent years, it has been introduced to the recreational drug arena where it is consumed intentionally or inadvertently via usage in pharmaceutical opioid mimicking products, as a heroin cutting agent or as a complete heroin substitute. It has the expected opioid effects including euphoria, drowsiness, miosis, and respiratory depression. Studies suggest that acetyl fentanyl is 5 to 15 times more potent than heroin.

Objective: To successfully analyze acetyl fentanyl in postmortem blood by liquid chromatography-tandem mass spectrometry (LC-MS/MS), and to evaluate the first reported incidence of acetyl fentanyl in Georgia for demographic and illicit factors.

Method: Utilizing the laboratory's current extraction procedure for basic drugs, acetyl fentanyl was isolated from the biological matrix by acetone precipitation. Subsequently, the samples were analyzed by LC-MS/MS. The method quantitated acetyl fentanyl at a linear calibration range from 2.5-40 µg/L. The lowest non-zero calibrator, 2.5 µg/L, was determined to be the lower limit of quantitation. The limit of detection was 0.5 µg/L. Acetyl fentanyl was also added to the postmortem comprehensive screen, enabling the acquisition of relative retention time and mass spectra. Acetyl fentanyl was identified and quantitated in 4 cases from Georgia that were submitted within a short 2 month-time span. A detailed study of each case was then performed to investigate the circumstances surrounding the death, route of administration, and other drugs that were detected in combination with acetyl fentanyl.

Result: The Toxicology section of the Georgia Bureau of Investigation crime laboratory received 4 cases from late November 2015 to January 2016 that were determined to contain acetyl fentanyl. Heroin was suspected for 2 of the cases, and illicit drugs use was suspected for the others. The acetyl fentanyl blood concentrations ranged from 3.5 to 360 µg/L. The decedents were both male and female, Caucasian and Asian, with ages ranging from 29-34 years old. Three of the decedents were found at home with history of drug abuse, while one Asian male decedent was found unresponsive at a party with friends. The cause of death for the case with the reported 360 µg/L of acetyl fentanyl was determined to be acute acetyl fentanyl toxicity; the others were combined toxicity with other drugs including fentanyl, cocaine, 3,4-methylenedioxymethamphetamine (MDMA), alprazolam, citalopram, and ethanol. Two cases with lower quantitation of acetyl fentanyl (3.5 and 4.8 µg/L) were also positive for fentanyl. Neither heroin nor its metabolite 6-acetyl morphine (6-AM) were detected in blood or urine.

Conclusion/Discussion: Since 2013, acetyl fentanyl has been reported in North Carolina, Rhode Island, West Virginia, Louisiana, Maryland, and Florida. At the GBI, suspected heroin cases were submitted, but acetyl fentanyl was detected. Analysis using LC-MS/MS was validated to be a sensitive and specific method for the detection and quantitation of acetyl fentanyl in postmortem blood. Acetyl fentanyl was detected in a wide range of concentrations, and in combination with many different drugs including fentanyl.

Keywords: Acetyl Fentanyl, LC-MS/MS, Method Validation

P38

Deadly Hit – The Explosion of Illicit Fentanyl and Fentanyl Analogue Deaths in Miami-Dade County, FL

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

Background/Introduction: Towards the end of 2014, the Miami-Dade County Medical Examiner Department Toxicology Laboratory began to notice an increase in the number of fentanyl positive postmortem cases. There was no history of fentanyl prescriptions in these cases, rather these were deaths of heroin addicted individuals. Analysis of drug paraphernalia confirmed our suspicions that heroin was being tainted with fentanyl and its analogues. It was this illicit supply of fentanyl flooding the market that was responsible for the deaths, and not the use of legally prescribed pharmaceutical fentanyl.

Objective: To present all illicit fentanyl and fentanyl analogue cases from 2014 to present, and identify trends in regards to cause and manner of death and other drugs detected. Demographic information will also be included in this study.

Method: The Miami-Dade County Medical Examiner Department (MDME) processes approximately 3600 cases per year. All case data is cataloged in LIMS which captures demographic information, terminal event data, social and medical history, toxicology results, and autopsy reports. Utilizing the case information from LIMS, data will be presented in a visual manner to compare and contrast the illicit fentanyl and fentanyl analogue positive deaths. Cases involving fentanyl patches or other medicinal fentanyl will not be included. In order to ensure that no earlier cases had been missed, all heroin positive cases from 2013 through the end of 2014 were screened by ELISA for fentanyl. All cases that screened positive for fentanyl were quantified by GC/MS (LOD = 0.5 ng/mL, LOQ = 1.0 ng/mL).

Result: Since 2014, illicit fentanyl and/or fentanyl analogues have been detected in 178 cases. There were no cases in 2013. The following table displays the number of cases in which fentanyl and/or fentanyl analogues were detected by year.

Year	Fentanyl	Fentanyl & Acetyl Fentanyl	Acetyl Fentanyl	Fentanyl & β-hydroxythiofentanyl	β-hydroxythiofentanyl
2014	13	0	0	0	0
2015	74	4	0	5	3
2016 to date	70	8	1	0	0

Concentrations of fentanyl ranged from <1 ng/mL to 111 ng/mL, with a mean concentration of 15 ng/mL and a median of 11 ng/mL. White males comprised 71% of all cases, 18% of the cases involved white females, followed by black males at 9% and 2% were black females. Decedents ranged in age from 17 years to 65 years, with a mean age of 37 years and a median of 36 years. Evidence of heroin use was detected in 66% of the cases, 64% were positive for cocaine and/or cocaine metabolites and alprazolam was found in 35% of cases. 96% of the deaths were ruled as accidental drug overdoses.

Conclusion/Discussion: Miami-Dade County has seen a large growth in the number of accidental overdose cases in which fentanyl was detected. The abundance of cheap fentanyl and fentanyl analogues on the black market has led to mass adulteration of street drugs of different types, most commonly heroin and cocaine, but also fake Xanax pills. This in turn has led to a huge increase in the number of overdose deaths in Miami-Dade County and across the country. Unfortunately this epidemic appears to be far from over; in the month of April 2016 the MDME detected illicit fentanyl in 22 decedents which is the highest number of cases per month to date.

Keywords: Case Study, Fentanyl, Opioids

P39

Urine Naloxone Levels in Patients Prescribed Combination Dosage Forms: Buprenorphine/Naloxone

Oneka Cummings*, Frank Wallace, Erin Strickland, Gregory L. McIntire; Ameritox, LLC, Greensboro, NC

Background/Introduction: Combination dosage forms of buprenorphine and naloxone (e.g., Suboxone®, Zubxolv®, and Bunavail®) are prescribed in drug addiction treatment. These formulations include naloxone to prevent diversion of buprenorphine for intravenous injections and illicit use, and to negate the effects of opiate overdose. Given the relatively short half-life of naloxone in plasma and the noted poor bioavailability via sublingual or buccal administration, it is commonly thought that detection of naloxone in the urine of these patients is less reliable than detection of buprenorphine and norbuprenorphine. However, a review of several thousand patient results demonstrates that naloxone is present in the urine of these patients at a higher rate than previously reported, and at relatively high levels. These data are relevant to clinical decisions based on drug monitoring results.

Objective: The objective of this work was to determine the frequency and level of naloxone post dosing of combination buprenorphine/naloxone products.

Method: Human urine samples from individuals prescribed either Suboxone®, Zubxolv®, or Bunavail® were tested for buprenorphine, norbuprenorphine and naloxone using a conventional liquid chromatography/tandem mass spectrometry (LC/MS/MS) method. This method was developed and validated using a Waters Aquity TQD LC/MS/MS system and a Restek Raptor™ Biphenyl Column, 2.7 µm, 50 x 2.1 mm. Samples are first hydrolyzed for 30 min at 60°C using recombinant beta-glucuronidase (IMCSzyme®) and then extracted by solid phase extraction (SPE) using SPEware CEREX® HPSCX, 1 mL NBE Column, before reconstitution with mobile phase and injection on the LC/MS/MS. Buprenorphine, norbuprenorphine, and naloxone standards were obtained from Cerilliant. The column temperature was 50°C and the cycle time for the method was 3.3 minutes.

Result: Analysis of 7229 patient samples demonstrated the presence of naloxone is as much as 91.9% of Suboxone® patients, 94.5% of Zubsolv® patients, and 80.9% of Bunavail® patients tested for naloxone independent of dose as shown in table 1. All three formulations demonstrated > 98% positive for buprenorphine (i.e., buprenorphine and/or norbuprenorphine). Observations on positivity rates for buprenorphine, norbuprenorphine, and naloxone in a small population have been previously reported by Tzatzarakis et al in their 2015 J. Anal. Toxicol. Publication. Naloxone is negative more frequently than buprenorphine suggesting that nonadherence is not the principle cause for naloxone negative results; rather metabolic differences, dose level, and inability to detect levels of naloxone below 10 ng/mL. These data demonstrate that positive naloxone results are expected from the analysis of most patient urine post dosing combination forms of buprenorphine and naloxone, but naloxone is not a definitive indicator of adherence to medication regimens.

Table 1. Positivity rates for 7229 Buprenorphine-Naloxone patient samples

Medication	Total Prescriptions	% Positive Buprenorphine	% Negative Buprenorphine	% Positive Naloxone	% Negative
Suboxone ~4BUP:1LOX	5746	98.7%	1.3%	91.9%	8.1%
Zubsolv ~4BUP:1LOX	687	99.6%	0.4%	94.5%	5.5%
Bunavail ~6BUP:1LOX	47	97.9%	2.1%	80.9%	19.1%

Conclusion/Discussion: Naloxone positives are present at or above 90% of samples for conventional formulations and at or above 80% for the lower concentration Bunavail® formulation in post dose tests. Urine medication monitoring continues to be a reliable tool for monitoring compliance with buprenorphine/naloxone dosage forms. It is important that medical professionals be knowledgeable of current testing technology and its application to interpretation of test results.

Keywords: Suboxone, Buprenorphine, Naloxone

P40 WITHDRAWN

P41

Age and Gender Trends in Broad Spectrum Liquid Chromatography Tandem Mass Spectrometry-Based Urine Drug Screening Positivity Rates in Ontario Canada

Adam S. Ptolemy*; Dynacare, London, Ontario, Canada

Background/Introduction: Comprehensive and specific information regarding trends in the prevalence and demographics of drug use within a specific region is often not widely available. Urine drug screening positivity rates for a defined population within a specific geographical area has recently been used to obtain this information, but no such studies have been published using a cohort of Canadian subjects.

Objective: Identify time, gender and age-specific trends in qualitative broad spectrum liquid chromatography tandem mass spectrometry (LC-MS/MS) based urine drug screening positivity rates for subjects tested within a healthcare setting in Ontario, Canada.

Method: LC-MS/MS urine drug screening results from approximately 114,000 female and 160,000 male patients, tested between January 1, 2014 and December 31, 2015, were retrospectively reviewed. All urine specimens underwent β-glucuronidase enzymatic hydrolysis and protein precipitation prior to testing. Screening was performed using a Waters Acquity liquid chromatography system paired with a Waters Xevo TQD triple quadrupole mass spectrometer operated in positive-ion electrospray ionization mode (+ESI). This LC-MS/MS testing protocol identified the presence of drugs within the following drug classes: anesthetic (ketamine); anticonvulsant (gabapentin); antidepressant (bupropion, trazodone); benzodiazepine (alprazolam, clonazepam, diazepam, flunitrazepam, flurazepem, lorazepam, nitrazepam, oxazepam, phenazepam, temazepam, triazolam); cannabinoid; opioid (buprenorphine, codeine, fentanyl, hydrocodone, hydromorphone, morphine, naltrexone, meperidine, methadone, oxycodone); stimulant (amphetamine, methylphenidate); and illicit (benzylpiperazine, cocaine, heroin, mephedrone, methamphetamine, MDPV, MDEA, MDMA). Relevant drug metabolites and related compounds (naloxone, levamisole) were also included in this test. A total of 63 different compounds were screened using their respective positive/negative cut-off concentrations. The positivity rates for all analytes were tabulated and partitioned by month of testing, gender and age (≤19, 20 to 29, 30 to 39, 40 to 49, 50 to 59, 60 to 69, 70 to 79 and ≥80 y).

Result: The highest respective male and female positivity rates for selected licit and illicit drugs are presented in the table below. Cannabinoid, cocaine, heroin, methadone and buprenorphine use was highest amongst the male and female 20 to 29 y cohorts and consistently decreased with age. Male and female positivity rates for codeine, morphine, oxycodone, gabapentin, alprazolam, temazepam, oxazepam and lorazepam all consistently increased with age. Amphetamine (p = 0.0003) and methamphetamine (p = 0.0002) use increased significantly from 2014 to 2015. Methamphetamine positivity rates in the female age cohorts ≤49 y and the male age cohorts 20 to 69 y increased during this time period. Levamisole, a cocaine cutting agent, was detected in the majority of benzoylecgonine positive specimens. All positivity rates of benzylpiperazine, mephedrone, MDPV, MDEA and MDMA were <0.2%.

Drug or Drug Metabolite	Males		Females		
	Age Range (y)	Positivity Rate (%)	Age Range (y)	Positivity Rate (%)	
6-Acetylmorphine	30 to 39	1	20 to 29	1	
Benzoylecgonine	20 to 29	14	20 to 29	13	
Norbuprenorphine	20 to 29	11	20 to 29	16	
7-Aminoclonazepam	40 to 49	7	40 to 49	11	
Norfentanyl	20 to 29	9	20 to 29	8	
Levamisole	20 to 29	9	20 to 29	10	
Methadone/EDDP	20 to 29	47	20 to 29	48	
Methamphetamine	30 to 39	7	20 to 29	5	
Methylphenidate	≤19	5	30 to 39	4	
THCA	20 to 29	47	20 to 29	33	

Conclusion/Discussion: A review of broad spectrum urine toxicology screening results identified clear gender and age trends in both licit and illicit drug use. The 20 to 29 y age cohort had the relatively highest overall prevalence of illicit drug use. Fentanyl use was also most prevalent in this cohort. Use of methamphetamine was the only illicit drug to significantly increase over the studied time period.

Keywords: LC-MS/MS Urine Drug Testing, Positivity Rate, Demographic Trends

P42
Substantiating Mechanistic Inhibition of Buprenorphine Metabolism

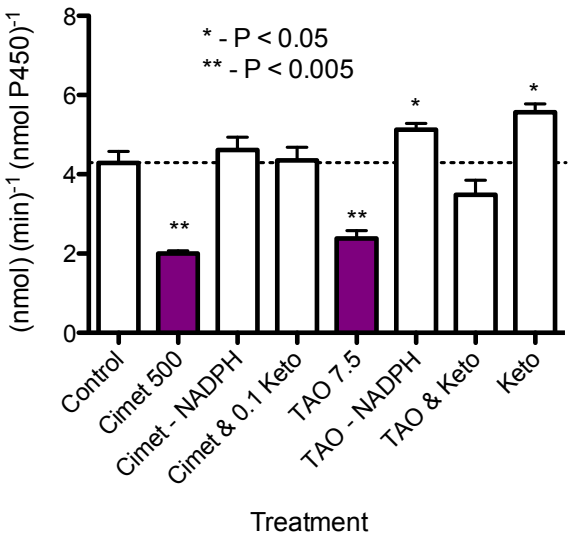
Caleb Ham*, Wenfang B. Fang, Yuequiao Fu, David E. Moody; Center for Human Toxicology, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT

Background/Introduction: Time-dependent inhibition (TDI) can be accurately described as inhibition arising from pre-incubation of the inhibitor with the enzyme under study and its necessary co-factors. When TDI results in direct injury to the enzyme, it is referred to as mechanism-based inhibition (MBI). MBI of metabolism of the opioid buprenorphine was studied in order to shed light on possible drug-drug interactions with the H_2-receptor antagonist cimetidine and the proton pump inhibitor (PPI) rabeprazole. Buprenorphine is a mixed narcotic agonist-antagonist used to treat opioid dependence; it is also widely used as an analgesic with diversion potential. H_2-receptor antagonists and PPIs are commonly prescribed to reduce gastric acid production. Drug-drug interactions may occur when buprenorphine and a drug are taken simultaneously, inhibiting the body's cytochrome P450s (CYP450) from metabolizing the drugs into sub-toxic levels. The CYP450 family enzymes are xenobiotic metabolizing enzymes located in the liver, small intestine, and large intestine. These enzymes are mainly comprised of a polypeptide chain and heme cofactor. Once the heme binds with oxygen, cytochrome P450 utilizes NADPH as an H+ energy source for oxidation of buprenorphine via the NADPH-cytochrome P450 oxidoreductase. Therefore, NADPH acts as the energy source for studying the drug interactions of buprenorphine and potential inhibitors. Previous results from this lab have shown that both cimetidine and rabeprazole display kinetics of inhibition of buprenorphine that are coincident with MBI.

Objective: Our objective was to substantiate MBI of CYP450-dependent metabolism of buprenorphine by cimetidine and rabeprazole.

Method: Dual incubation procedures were utilized in order to identify potential MBI. In the primary incubations, recombinant CYP450 3A4 (CYP3A4), the test inhibitor or a positive control for MBI of CYP3A4, troleandromycin (TAO), and an NADPH source were pre-incubated in phosphate buffer. At 15 minutes, aliquots were taken and diluted 10-fold into a secondary incubation system containing phosphate buffer, substrate and additional NADPH source to measure the enzyme's % remaining activity. The negative control contained no inhibitors and was used as 100% activity. The CYP3A4-dependent nature of inhibition was then ascertained by removing NADPH or by addition of a CYP3A4 selective reversible inhibitor, ketoconazole. Norbuprenorphine production from buprenorphine N-dealkylation was measured using liquid-liquid extraction and liquid chromatography-tandem mass spectrometry (LC-MSMS).

Result: The figure illustrates the effect of cimetidine, TAO, and ketoconazole when pre-incubated with a CYP3A4/ NADPH generating system and diluted into a second incubation system containing the substrate buprenorphine and additional NADPH generating system. The control solution contained CYP3A4, the NADPH source, and phosphate buffer. Pre-incubation with cimetidine and TAO showed significant inhibition of activity. Removal of NADPH eliminated all inhibition. This exemplifies the need of an NADPH source for irreversible inhibition to occur within in vitro settings. Co-incubation with the reversible CYP3A4 inhibitor 0.1 mM ketoconazole also eliminated inhibition by both cimetidine and TAO. With only ketoconazole in the primary incubation there was no inhibition in the secondary system. This established that the dilution of the aliquot was sufficient to reduce ketoconazole to a non-inhibiting concentration. Similar results were obtained when rabeprazole was used in place of cimetidine.



Conclusion/Discussion: These results provide compelling evidence for MBI. The toxicological importance of MBI is the potential for prolonged inhibition of the enzyme as reversal of inhibition requires not just elimination of the inhibitor, but also regeneration of the enzyme. Other inhibitors of buprenorphine N-dealkylation (e.g., delavirdine) have been found to result in increased plasma buprenorphine during in vivo inhibitions. This implies that alternate routes of metabolism (i.e., glucuronidation) cannot fully compensate for the reduced oxidative metabolism. As buprenorphine, more so than its metabolites, is the main active agent in the central nervous system (CNS), inhibition of norbuprenorphine formation can lead to greater exposure of the CNS to buprenorphine and thereby enhance potential for respiratory depression and other adverse CNS effects.

Funding: This research was supported by grants from the National Institute of Justice, 2011-DN-BX-K532 and 2015-IJ-CX-K009.

Keywords: Mechanism-Based Inhibition, Buprenorphine Metabolism, Drug Interactions

P43
LC-MS/MS Method for the Analysis of Miscellaneous Drugs in Wastewater During Football Games III

Brandon Stamper*[1,2], Waseem Gul[1,3], Murrell Godfrey[2], Shahbaz W. Gul[1], Mahmoud A. ElSohly[1,3,4]; [1]ElSohly Laboratories, Inc., Oxford, MS, [2]Department of Chemistry and Biochemistry, [3]National Center for Natural Products Research, and [4]Department of Pharmaceutics and Drug Delivery, University of Mississippi, University, MS

Background/Introduction: Continuing our previous studies analyzing drugs of abuse in municipal wastewater (*LC-MS-MS Method for Stimulants in Wastewater During Football Games* and *LC-MS-MS Method for Analysis of Opiates in Wastewater During Football Games II*), a method was developed for the analysis of miscellaneous drugs of abuse in wastewater samples using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Eight drugs and metabolites were analyzed including EDDP, fentanyl, norfentanyl, meperidine, normeperidine, methadone, phencyclidine, and tramadol. These drugs were chosen because of their widespread abuse.

Objective: The purpose of this experiment was to develop a method for analyzing EDDP, fentanyl, norfentanyl, meperidine, normeperidine, methadone, phencyclidine, and tramadol in wastewater, and to apply this method to analyze wastewater samples collected during college football games.

Method: A method was developed and validated utilizing silica-based mixed-mode C8 and cation exchange solid phase extraction (SPE) and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) for the analysis of miscellaneous drugs of abuse in wastewater. Wastewater samples were collected at both the Oxford Waste Water Treatment Plant in Oxford, Mississippi (MS) and the University Wastewater Treatment Plant in University, MS. These wastewater samples were collected on weekends in which the Ole Miss Rebel football team held home games (Vaught-Hemingway Stadium, University, MS 38677). The LC-MS/MS method utilized multiple reaction monitoring (MRM) mode due to its high selectivity and specificity. Two MRMs were monitored (one MRM as quantifier and the other as qualifier).

Result: Validation of the analytical method showed method accuracy ranging from 89 to 110% with a relative standard deviation of 1.89 to 9.37% for low level positive control samples. Limits of detection were 0.075 ng/mL for all analytes. Limits of quantitation and linearity vary between analytes. Limits of quantitation ranged from 0.075 ng/mL to 2.5 ng/mL. ULOL ranged from 25 ng/mL to 100 ng/mL. The real wastewater samples were found to contain only tramadol in 25 samples at quantifiable levels (LOQ = 0.075 ng/mL; analyzed at concentrations ranging from 0.09 ng/mL to 1.62 ng/mL). Meperidine, normeperidine, and methadone were also detected, but were under the limit of quantitation (LOQ = 0.625 ng/mL for meperidine, normeperidine, and methadone).

Conclusion/Discussion: An LC-MS/MS method was successfully validated for the analysis of eight drugs in wastewater samples. The method was reproducible for all eight drugs and was applied to analyze wastewater samples collected from the University of Mississippi and City of Oxford around football game times. Tramadol was the most prevalent of the analyzed drugs, and showed a sizeable increase in concentration during two highly attended football games. This method can be applied to help law enforcement agencies to have real-time data on drug usage in local communities. For example, studying trends over time will help law enforcement determine whether current strategies are effective.

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

P44

Analysis of Aerosolized Methamphetamine Infused E-liquids by Solid Phase Microextraction Using Gas Chromatography Mass Spectrometry (SPME-GC-MS), Direct Analysis in Real Time AccuTOF™ Mass Spectrometry (SPME-DART-MS), and Headspace Gas Chromatography Mass Spectrometry (HS-GC-MS)

Rose I. Krakowiak*[1], Karen E. Butler[1], Joseph W. Stone[1], Tyson R. Baird[1], Justin L. Poklis[2], Joseph B McGee Turner[3], Alphonse Poklis[4], Michelle R. Peace[1]; [1]Department of Forensic Science, [2]Department of Pharmacology & Toxicology, [3]Department of Chemistry, [4]Department of Pathology, Virginia Commonwealth University, Richmond, VA

Background/Introduction: With the growing popularity of electronic cigarettes (e-cigs), the use of these devices has expanded from its traditional use as a nicotine delivery system to an illicit drug delivery system. The internet is filled with websites, blogs and forums teaching users how to modify their e-cigs to deliver an illicit drug while also maintaining optimal drug delivery of their device. With the exception of the flavoring agents, when e-liquids are "vaped," an odorless vapor is produced. Therefore, when e-liquids are formulated to contain illicit drugs, users can "vape" illicit drugs in public without detection.

Objective: The goal of this study was to develop a qualitative method of analyzing methamphetamine present in vapors produced by an electronic cigarette. Analysis of a lab-formulated e-liquid containing methamphetamine was conducted using Direct Analysis in Real Time AccuTOF™ Mass Spectrometry (DART-MS) and Gas Chromatography Mass Spectrometry (GC-MS).

Method: An e-liquid formulation was made to contain 120 mg/mL of methamphetamine in propylene glycol (PG) and vegetable glycerin (VG) in a ratio of 50:50 (v:v). The e-liquid was "vaped" from a typical electronic cigarette, a KangerTech AeroTank, 1.8 Ω preassembled atomizer, and an eGo-V2 at variable voltage battery at 4.3 V for 4 sec at 2.3 mL/min. A 100 µm Supelco, Inc. polydimethylsiloxane (PDMS) SPME fiber was used to extract the aerosolized drugs from a water trap for analysis by DART-MS and GC-MS. Analysis was performed using an Agilent GC/MS 6890N/5973 Mass Selective Detector instrument with a Restek HP-5MS column (0.25mm x 30 m x 250 µm). For GC-MS analysis, each sample was analyzed in splitless mode, with a 15 minute fiber desorption time. The initial temperature was set at 120 °C, with a ramp to 300 °C at 10 °C/min, and then a hold for 12 min, for a total run time of 30 min. For DART-MS analysis, the SPME fiber was directly exposed to the helium stream of a JEOL JMS T100LC AccuTOF™ Mass Spectrometer at 200 °C. Ion spectra were collected in positive ion mode at a setting of 20, 60, and 90 V for orifice one. Needle voltage was set to 3500 V, with the discharge electrode at 150 V, and the grid electrode at 250 V. The e-cig mouthpiece and coil were also assessed after vaping for any methamphetamine residue by Headspace Gas Chromatography Mass Spectrometry (HS-GC-MS). The mouthpiece and coil were removed from the electronic cigarette and placed QorpackTM Unlined Round Cans with Triple Tite Lid (32 oz/946.3 mL) containing an Albrayco Technologies Inc. charcoal strip hanging from the lid to collect the volatiles. The cans were sealed and placed in an oven at 60 °C for one hour. When removed from the oven, the charcoal strip was placed in a 20 mL scintillation vial with 2 mL carbon disulfide, vortexed and analyzed using the GC-MS parameters previously stated.

Result: SPME DART-MS and SPME GC-MS were fast and efficient analytical methods for analyzing aerosolized methamphetamine produced by an e-cig, demonstrating its ability to be vaped by users. HS-GC/MS is an effective tool for analyzing e-cig components.

Conclusion/Discussion: This study demonstrates an efficient and safe method to analyze the vapor produced by e-cigs and their components, particularly if they contain illicit substances.

Funding: This project was supported by Award No. 2014-R2- CX-K010, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice and National Institute on Health (NIH) Center for Drug Abuse grant P30DA033934. The opinions, findings, and conclusions or recommendations expressed in this publication/program/exhibition are those of the author(s) and do not necessarily reflect those of the Department of Justice.

Keywords: Methamphetamine, Electronic Cigarettes, E-Liquids

P45

Temperature Characterization of Electronic Cigarette Atomizer by Infrared Temperature Sensing

Haley Mulder*[1], Ivy P. Blue IV[1], Justin Poklis[2], Jesse Patterson[1], Rose Krakowiak[1]., Kaitlyn Forsythe[1], Jasmynn Royals[1], Alexandra Dupont[1], Alphonse Poklis[1,2,3], Michelle Peace[1], Joseph Turner[4]; [1]Department of Forensic Science, [2]Department of Pharmacology and Toxicology, [3]Department of Pathology, [4]Department of Chemistry, Virginia Commonwealth University, Richmond, VA

Background/Introduction: Electronic cigarettes (E-cigs) are marketed as an alternative to smoking cigarettes. The e-cig delivers nicotine to the user through an atomizer that consists of a wired coil and wicking system that is saturated with the refill formulation liquid (e-liquid). The coil becomes heated after the user activates a battery by either depressing a button or negative pressure produced by inhaling on the device. Propylene glycol (PG) and vegetable glycerin (VG) are used as humectants to deliver drug and flavors to the user. Users can vary the ratio of PG to VG, depending on their preference. Additional modifications that users make to the e-cigs to improve drug delivery are selection of different wire and wick dimensions, configuring the coils between simple and complex, and adjusting voltage to heat the coil.

Objective: The purpose of this study was to characterize the coils in the e-cig by measuring the maximum temperature outputs while saturated with e-liquid.

Method: The atomizer used was a Kayfun Lite Clone by Kangertech. The wires used were Kanthal A-1 and Nichrome 20:80 and the wire gauges ranged from 30-34 on the American Wire Gauge Scale. The coils were hand built as contact and non-contact configurations and wrapped so that the wire resistance had a measurement of 1.8 Ω. The temperature of the e-cig coils was measured using the thermoMETER CT M3 dual IR laser temperature sensor (Micro-Epsilon, Raleigh, NC) operated at 2.3µm. The detection range for the sensor was 100 ℃ to 600 ℃ and was recorded by Compact Connect version 1.9.8.6. The e-liquids used to characterize the temperature output were 100% propylene glycol and 100% vegetable glycerin from Wizard Labs, and contained no nicotine.

Result: Using 100% vegetable glycerin, as the voltage of the e-cig was increased, the temperature of the coils increased from 204 °C to 305 °C for all gauges and both types of wire wrapped in both configuration. Using 100% propylene glycol, the coils showed temperature ranges from 189 °C to 210 °C with increasing voltage for all gauges and both types of wire wrapped in both configuration. The difference in temperature outputs between the two liquids is due to their thermal conductivity. Vegetable glycerin has a higher thermal conductivity than propylene glycol at 0.258 W/m*K at 300 K where propylene glycol has a thermal conductivity of 0.147 W/m*K at the same temperature.

Conclusion/Discussion: Understanding how the two e-liquid components are heated by the e-cig coils can help to determine how e-liquids are aerosolized. Gauge and type of wire used will change the temperature profile for both the vegetable glycerin and the propylene glycol. An increase of voltage resulted in an increase of temperature which may result in an increase in the amount of e-liquid being aerosolized. The temperature ranges produced at the same voltages were higher for the vegetable glycerin than the propylene glycol which may result in more vegetable glycerin to be aerosolized than propylene glycol. The temperatures at which the e-liquid is heated can provide insight to the size of the aerosolized particles which impacts the bioavailability of the drugs in the e-liquid and other active ingredients they contain. This, therefore, will impact toxicity of the drugs inhaled.

Funding: This project was supported by Award No.2014-R2-CX-K010, 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 publication/program/exhibition are those of the author(s) and do not necessarily reflect those of the Department of Justice.

Keywords: Electronic Cigarettes, E-Liquid, E-Cig Coils

P46
The Effect of Light and Temperature on Electronic Cigarette E-Liquid Formulations

Katilyn N.L. Brooks*[1,3], Justin Poklis[2], Joseph B. McGee Turner[3], Alphonse Poklis[4], Michelle R. Peace[1]; [1]Department of Forensic Science, [2]Department of Pharmacology and Toxicology, [3]Department of Chemistry, [4]Department of Pathology at Virginia Commonwealth University, Richmond, VA

Background/Introduction: Nicotine is an air and light sensitive compound, commonly found in electronic cigarette refill formulations, or e-liquids. Lack of quality control in the manufacture of e-liquid products could lead to lack of stability and the production of secondary unintended components over time.

Objective: The objective of this study was to determine the stability of nicotine-containing e-liquids over nine months in different storage conditions where lighting, temperature, container type, and nicotine concentration were varied.

Method: Each combination of variables was run in n=6. Concentration of nicotine was determined by using a 3200 Q Trap attached to a SCL HPLC/MS/MS system. Chromatographic separation was performed on a Hypersil Gold 3x50mm, 5 µm column. The injection volume was 10 µL with a flow rate of 0.5 mL/min. The ionspray voltage was set to 5000 V with a declustering potential of 35 eV and the source temperature was 600 °C with 30 mL/min curtain gas flow. Ion source gasses 1 and 2 were set to 50 mL/min and 30 mL/min, respectively. The total run time for this method is 2 minutes and the instrument was operated in multiple reaction monitoring mode (MRM) monitoring the following *m/z* transitions: nicotine, 163>130 and 163>117; and nicotine-d4, 167>134. In glass containers, three lab-prepared formulations were evaluated at a concentration of 18 mg/mL nicotine: 100% propylene glycol (PG), 100% vegetable glycerin (VG), and 50:50 PG:VG. Also in glass containers, lab-prepared formulations with concentrations of 6 mg/mL and 36 mg/mL nicotine were evaluated in 50:50 PG:VG. Formulations with a concentration of 18 mg/mL in 50:50 PG:VG were evaluated in plastic and amber glass containers. Additionally, three commercial samples were evaluated in their original containers. All of the samples previously mentioned were stored in different environments: light and room temperature (RT), dark and RT, and dark and refrigerated at 4 ºC.
Separately, the effect of heat on commercial samples was also evaluated. Four different commercial samples in their original containers were stored in varying environments including light and heat (40 ºC), dark and heated, light and RT, and dark and RT.

Result: The concentration of nicotine decreased significantly from time 0 through time 12 weeks, and then plateaued for the remaining time when exposed to light conditions. For example, the light and RT 36 mg/mL sample decreased to 27.8 mg/mL in 12 weeks. The container type (glass, plastic, and amber glass) did not impact the concentration of nicotine in the sample. The concentration of nicotine in commercial samples deteriorated from 20.5 mg/mL to 13.2 mg/mL in 12 weeks and then plateaued. No statistically significant different between light and RT, dark and RT, and dark and refrigerated exists.

Conclusion/Discussion: Understanding how nicotine degrades in e-liquid samples can help the consumer determine the best plan of action when determining how to store their e-liquids and it can help the manufacturer determine shelf life of their product. Also, if electronic cigarettes are being used as a nicotine replacement therapy, then degradation of the nicotine in the e-liquids could cause this to be an ineffective method of quitting tradition tobacco products.Additionally, defining the degradation of e-liquids will ultimately lead to analyses to define the formation of potential biomarkers that can be used for forensic toxicological investigations.

Keywords: Electronic Cigarette, E-liquid, Nicotine Stability

P47

A Presumptive Evaluation of Commercial Refill Formulations for Nicotine by a Microchemical Analysis Method Developed as a Field Test

Laura A. McNew*[1], Justin L. Poklis[2], Joseph B. McGee Turner[3], Alphonse Poklis[4], Michelle R. Peace[1]; [1]Department of Forensic Science, [2]Department of Pharmacology & Toxicology, [3]Department of Chemistry, [4]Department of Pathology, Virginia Commonwealth University, Richmond, VA

Background/Introduction: While cigarette use by minors has steadily declined over the past five years, electronic cigarette (e-cigarette or e-cig) use has increased at an alarming rate. The use of e-cigarettes tripled from 2013 to 2014 among middle school and high school students. Unenforced rules and regulations, misinformation, low cost, high availability, and youth-focused marketing tactics have all attributed to the increasing appeal of this new fad to minors. Due to the lack of Food and Drug Administration (FDA) regulations on the sale and use of e-cigs, it has become increasing difficult to prevent the sale of these devices to minors unless regulated by states, cities, and/or jurisdictions.

As of December 2015, at least 48 states and 2 territories have prohibited the sale of e-cigarettes to minors. Even so, minors can still easily obtain e-cigarette devices and purchase e-liquids containing nicotine. A few police agencies have reported conducting interventions with retail outlets to stop the sale of e-liquids to minors. Because these devices are also used to smoke nicotine-free flavored liquid, it is necessary to have a presumptive test for nicotine available to law enforcement to create community educational opportunities and to potentially prevent crime labs from being inundated with e-cigarette devices and e-liquids.

Objective: The purpose of this research project was to develop a presumptive, colorimetric method that can be easily used by law enforcement to evaluate commercial e-liquids.

Method: Four colorimetric methods (Modified Sanchez, Meltzer, Zwikker, and Bromothymol Blue) described in the literature for the detection of nicotine were evaluated for sensitivity and specificity. A concentration of 99% nicotine was first used to assess each method for ease of use and distinctive coloring. A 1.2% solution of nicotine was used to assess sensitivity. Since only the modified Sanchez color test using Meltzer's reagent and Zwikker's reagent showed a positive color reaction when exposed to 99 % nicotine, interference studies were performed using tea and a compound containing aspirin, acetaminophen, and caffeine. Tea and the aspirin/acetaminophen/caffeine compound were identified in previous literature as causing a false positive test for nicotine using Zwikker reagent. A preliminary LOD assessment was made with nicotine in methanol.

Result: Only the Zwikker reagent, commonly used to test for barbiturates, continued to give a positive test for nicotine when diluted to 0.15% in methanol. Neither tea, nor the aspirin/acetaminophen/caffeine compound, indicated an interference with the Zwikker. Thirty-two commercial e-liquids, varying in nicotine and caffeine concentrations, were tested using Zwikker reagent. While Zwikker was a positive indicator for nicotine, several false positives and false negatives were identified. Five e-liquids gave false positive results, three of these samples are known to contain either caffeine or melatonin. Three of 32 e-liquids with verified nicotine concentrations gave false negative results. Three of 32 samples with verified nicotine concentrations were "undetermined", due to the dark coloring of the e-liquid making a color change difficult to ascertain. Fifteen of 32 samples screened positive for nicotine in verified nicotine e-liquids.

Conclusion/Discussion: Zwikker reagent can be used to presumptively assess the presence of nicotine, but with some reservation. As with any field test for a drug, confirmatory testing should be performed.

Funding: This project was supported by Award No. 2014-R2-CX-K010, 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 publication/program/exhibition are those of the author(s) and do not necessarily reflect those of the Department of Justice." This statement shall appear on the first page of written publications.

Keywords: Nicotine Field Test, E-Cigarettes, Nicotine Color Test

P48

Passive Extraction of Nicotine from Components of Electronic Cigarettes Using Charcoal Strips

Ivy P. Blue IV*[1], Karen E. Butler[1], Joseph B. McGee Turner[2], Michelle R. Peace[1]; [1]Department of Forensic Science, [2]Department of Chemistry, Virginia Commonwealth University, Richmond, VA

Background/Introduction: With the growing popularity of electronic cigarettes (e-cigs), concern for their use as a method for consuming illicit or alternative substances has also grown. Currently, a safe and efficient method for the analysis of e-cigarettes and their e-liquid formulations in forensic science laboratories has not been defined in the literature. The adoption of a method used for fire debris analysis using passive extraction headspace methodology could be utilized for parts of electronic cigarettes, such as the atomizer containing the e-liquid and the wick.

Objective: The purpose of this study was to develop a safe analytical method using headspace analysis gas chromatography mass spectrometry (GC-MS) and passive extraction. This method takes advantage of the volatile nature of the e-liquid components utilizing technology available in most forensic laboratories.

Method: Qorpack™ Unlined Round Cans with Triple Tite Lid (32 oz/946.3 mL) with Albrayco Technologies Inc. charcoal strips hanging from the lid witha magnet and a paper clip were used to collect volatiles. A 5 cm long, 2.5 mm o.d. silica wick with a range of 25-500 µL of 6 mg/mL (0.15- 3 mg) nicotine in 50:50 propylene glycol: vegetable glycerin (PG:VG) was placed at the bottom of the can. The can was sealed and placed in an oven at 60 °C overnight, at 2 hours, and at 1 hour. After the allotted time, the cans were removed from the oven and the strip was placed into a 20 mL scintillation vial with 2 mL of carbon disulfide. After vortexing, a sample was transferred to a GC vial for analysis. A 6890N Agilent GC coupled with a 5973 MS, with an Agilent HP-5MS 30 m x 250 µm x 0.25 µm column was operated with MSD ChemStation E.01.01.355. The oven temperature program was 40 °C with a 2 minute hold, ramping to 300 °C at 27 °C per minute with a final 3 minute hold. The instrument was operated in split mode (60:1). Nicotine was identified using the NIST 14 database.

Result: Nicotine was observed at a retention time of 7.5 minutes for all volumes of the 6 mg/mL nicotine in 50:50 PG:VG. Nicotine was also detected with the shortest passive extraction time of 1 hour and at the lowest amount of nicotine at 0.15 mg on the wick, and responses were linear across the nicotine range (r^2>0.9875). Using only the wick from the e-cig, the nicotine was successfully detected. This amount is relevant as it could be similar to the residual drug remaining on a wick after a user has vaped.

Conclusion/Discussion: The passive extraction method is a safe and efficient method for drugs of interest from the components of an e-cigarette.

Keywords: E-Liquid, Headspace, E-Cigarettes, Nicotine

P49

The Proof is in the Powder - Deadly Mixtures on the Streets of Miami-Dade

Alexander D. Giachetti*, Diane M. Boland, George W. Hime; Miami-Dade County Medical Examiner, Miami, FL

Background/Introduction: Often overlooked in postmortem death investigation is drug paraphernalia found in close proximity to the decedent at the time of the terminal event. In many circumstances, syringes, spoons, rocks, and powders are found within arm's reach of the decedent. Not only does the presence of such paraphernalia imply recent use, it can also provide valuable information concerning drug trends in the local community. Due to the rise in designer drugs in the last several years, the Miami-Dade Medical Examiner Department (MDME) Toxicology Laboratory partnered with local law enforcement agencies to ensure that evidence collected at the scene is submitted to our laboratory. Suitable exhibits are analyzed in order to compliment toxicology testing and, more importantly, identify the drug or drug combinations that contributed to the death of individuals in Miami-Dade County. Consequently, analysis of the powders in particular has proved valuable in the identification of various deadly drug mixtures. Powders represent a probative piece of evidence that clearly displays the drugs consumed, as opposed to syringes and spoon residues that could be contaminated by multiple uses and users.

Objective: The objective of this study is to display a summary of results from powder evidence received and analyzed by the MDME Toxicology Laboratory from January 2015 to present.

Method: The fifty-nine powder exhibits selected for this study were analyzed using dilute and shoot sample preparation followed by gas chromatography coupled to a mass-spectrometer (GC-MS). The results were surveyed by utilizing the Laboratory's Information Management System (LIMS) and will be displayed for visual review.

Result: Fifty-nine powder exhibits from forty-two cases since January 2015 were identified as suitable for this study. A total of thirty-two powder exhibits contained heroin (54% of total samples) in a range of colors from pure white to brown. Of these powders, 62% of them were heroin and fentanyl, 38% contained heroin and cocaine, and 25% comprised of heroin, fentanyl, and cocaine.
The other twenty-seven powders consisted of a sole drug with or without fillers or cutting agent. While the majority of these were cocaine, less than 10% of the remainder consisted of solely heroin or fentanyl.
THC and Oxycodone were also identified in a small percentage of the mixtures.

Conclusion/Discussion: As indicated by the data, the combination of heroin and fentanyl dominates the streets of Miami-Dade County. In postmortem cases where powder evidence was submitted, the cause of death was solely attributed to toxicity from this deadly mixture. 50% of the powder evidence received this year contains heroin and fentanyl – proving that the combination is on the rise and continues to be an epidemic in our community. This study suggests that it is near impossible to buy illicit heroin without a mixture of fentanyl, cocaine, or both, possibly without the drug user knowing. Even after a lifetime of opiate abuse, no user is safe from Miami-Dade's current drug landscape.

Keywords: Seized Drugs, Cocaine, Heroin, Fentanyl

P50

A Retrospective Analysis of Methamphetamine Positive Driving Under the Influence Cases in Montana

Elizabeth Smalley*, Michelle Duffus, Scott J. Larson; Montana Department of Justice, Forensic Science Division, Missoula, MT

Background/Introduction: Methamphetamine is a commonly abused central nervous system stimulant, whose presence has risen steadily from 2009 to 2015 in forensic toxicology casework in the state of Montana. Over these seven years, Montana has observed a significant increase in methamphetamine-positive DUI (driving under the influence) cases, with drivers displaying a wide range of behaviors.

Objective: We present a cumulative analysis of methamphetamine-positive DUI cases submitted to the Montana Department of Justice Forensic Science Division from 2009-2015. Information includes number of cases and blood concentrations. In order to highlight the variability in observed impairment indicators associated with methamphetamine/amphetamine-only cases, DRE (Drug Recognition Expert) cases for 2015 are summarized.

Method: Whole blood samples were collected by law enforcement agencies from drivers suspected of driving under the influence. They were packaged in gray top tubes containing potassium oxalate/sodium fluoride and sent to the Forensic Science Division for analysis. Since 2013, only samples that had a blood ethanol level less than 0.10 g/dL were analyzed for drugs of abuse and prescription drugs on previously validated methods according to laboratory procedure using ELISA, GCMS, and LCMS. Prior to 2013, analysis was completed on all DUI samples where drugs were suspected, regardless of blood ethanol concentration. The total number of cases and corresponding blood concentrations were collected for the last seven years on methamphetamine/amphetamine-positive DUI cases. DRE information was also collected for cases in 2015. Information included the subject's performance on walk-and-turn (WAT), one leg stand (OLS), and modified Romberg balance test, as well as their assessment for horizontal gaze nystagmus (HGN), lack of convergence (LOC), pupil size, reaction to light, pulse, blood pressure, body temperature, and muscle tone.

Result: In 2009, the Montana Department of Justice Forensic Science Division received 23 methamphetamine-positive DUI cases. In 2015, that number jumped to 292, including 52 cases with DRE evaluations. During that timeframe the mean concentration rose from 0.166 mg/L to 0.364 mg/L (119% increase). Of 52 DRE cases, 18 had methamphetamine/amphetamine only and 34 contained other drugs in addition to methamphetamine/amphetamine, such as CNS depressants (14), cannabinoids (26), narcotic analgesics (7), and ethanol (5). Most predominantly, cannabinoids (THC and/or THCCOOH) were present in 26 of 34 mixed-drug cases. Ethanol was detected in only 5 of 34 mixed-drug cases, with one case being detected over 0.08 g/dL. It is worth noting that DRE officers are typically not contacted to do evaluations in cases that involve significant levels of ethanol. Varying impairment indicators were noted by DRE officers in the 18 methamphetamine/amphetamine-only cases: WAT (score 0-6), OLS (score 0-3), Romberg test (12-48 seconds), HGN (present in n=4), LOC (present in n=9), varying pupil size and reaction to light, normal to elevated pulse and blood pressure, normal to decreased body temperature, and normal to rigid muscle tone.

Conclusion/Discussion: Methamphetamine presence in Montana is rising, which is leading to an increased number of DUI cases involving methamphetamine/amphetamine, as well as an increase in blood concentrations. DRE observations varied greatly, presumably due to differences between stimulant and withdrawal symptoms of methamphetamine, differences in individual reactions to methamphetamine/amphetamine, and differences in blood concentrations. The observed impairment indicators were not concentration dependent. When evaluating impairment, the totality of the circumstances is necessary to identify the CNS stimulant category caused by methamphetamine/amphetamine use.

Keywords: Methamphetamine, DUID, DRE

P51
Field Evaluation of a New Homogeneous Buprenorphine Immunoassay

Michael Böttcher*, Nicky Günther; MVZ Labor Dessau GmbH, Dessau, Germany

Background/Introduction: Buprenorphine is a partial μ-agonist opioid with both agonist and antagonist properties which gained widespread use in substitution therapy of opiates dependent patients.. The commonly prescribed drugs are Subutex® and Suboxone® which is a combination of buprenorphine and naloxone. Buprenorphine is metabolized into norbuprenorphine, and both buprenorphine and norbuprenorphine are conjugated to glucuronide to become buprenorphine-glucuronide and norbuprenorphine-glucuronide. The currently available CEDIA® Buprenorphine assay detects Buprenorphine and Buprenorphine glucuronide.

Objective: The objective of this study was to evaluate a new Buprenorphine immunoassay currently in development that can detect buprenorphine, norbuprenorphine and their conjugated metabolites in human urine.

Method: The CEDIA® Buprenorphine II assay in development was compared to the on market CEDIA® Buprenorphine assay and liquid-chromatography tandem mass-spectrometry (UPLC-MS/MS). The immunoassays were run on the Beckman AU680 chemistry analyzer. Urine samples (n = 1119) were obtained from 921patients in opiate maintenance therapy for routine urine drug testing including Buprenorphine. CEDIA Buprenorphine assay was obtained from Thermo Fisher Scientific, and Buprenorphine II Reagents, Calibrators and Controls were supplied by Thermo Fisher Scientific for field evaluation. The CEDIA Buprenorphine assay uses a 5 ng/mL cut-off with 3 and 7 ng/mL controls. The Buprenorphine II assay uses a 10 ng/mL cut-off with 7.5 and 12.5 ng/mL controls. Positive samples in either CEDIA were analysed with a DIN EN ISO/IEC 17025 accredited UPLC-MS/MS method with and without prior enzymatic hydrolysis of the samples which allowed the quantification of buprenorphine- and norbuprenorphine-glucuronide. Total buprenorphine and norbuprenorphine was >5 ng/mL in 369 samples and 373 samples respectively.

Result: A modified CLSI 5-day precision was carried out on the CEDIA Buprenorphine II assay using the cutoff calibrator and controls demonstrated excellent precision. Method comparison study using 1119 urine samples demonstrated 99.2% overall correlation between CEDIA Buprenorphine II assay and LC-MS/MS, while CEDIA Buprenorphine assay showed 97.7% overall correlation compared to LC-MS/MS. When comparing the two immunoassays, there were 30 discrepant samples and 21 of these discrepant samples were detected as false positive on the CEDIA Buprenorphine assay due to cross-reactivity to codeine, morphine or tiapride. Four samples were false positive on the CEDIA Buprenorphine II assay, as confirmed by UPLC-MS/MS. Of the remaining 5 discrepant samples 3 samples had total buprenorphine and norbuprenorphine concentration between 5 and 10 ng/mL; and 2 samples were false negative by Buprenorphine assay due to high levels of norbuprenorphine and norbuprenorphine-glucuronide.

Conclusion/Discussion: The data demonstrate that the CEDIA Buprenorphine II assay has excellent correlation to LC-MS/MS, and has advantages over the current CEDIA Buprenorphine assay: reduced false positives due to reduced cross-reactivity to codeine, morphine and tiapride, and reduced false negatives due to improved detection of buprenorphine metabolites.

NOTE: The assay is currently in development and is not approved by the FDA.

Keywords: Buprenorphine, Metabolites, Immunoassay (CEDIA)

P52
Validation of Alpha-PVP/MDPV Immunoassay on Tecan Freedom Evo 75 with Randox ELISA Kit

Rebekah Boswell*[1], Mary S. Eley[2], Curt E. Harper[1]; [1]Alabama Department of Forensic Sciences, [2]University of Alabama at Birmingham, Birmingham, AL

Background/Introduction: Synthetic cathinones, colloquially known as "bath salts", are derivatives of the psychoactive alkaloid cathinone found in the Khat plant. These compounds produce stimulant effects similar to amphetamine by increasing dopamine levels in the brain. Alpha-pyrrolidinovalerophenone (α-PVP) and 3,4-methylenedioxypyrovalerone (MDPV) have been two of the most prevalent synthetic cathinones encountered in forensic toxicology casework over the past three years. α-PVP, also known as Flakka or gravel, has gained notoriety in the media due to reports of violent, bizarre, and dangerous behavior. Therefore, there is a need to develop a robust screening method. The Tecan Evo 75 using the Randox ELISA kit described herein provides a sensitive and quick immunoassay screening option in whole blood for both human performance (DUI) and postmortem applications.

Objective: To validate the Randox α-PVP/MDPV ELISA kit in 96 well format in whole blood using a semi-automated Tecan Freedom Evo 75 instrument.

Method: 96 well plates were coated with α-PVP antibody by Randox. Whole blood samples were diluted 1:50 with diluent (20 µL sample + 980 µL diluent). 50 µL of diluted sample was added to each well followed by 75 µL of horseradish peroxidase labeled antigen (conjugate) to allow for competitive binding. Incubation time with the antibody, enzyme substrate (TMB), and stop solution (HCl) were 30, 20, and 5 minutes, respectively. These times were monitored carefully to enhance reproducibility Optical density was measured at 450 nm by UV/VIS spectrophotometry. The cutoff for α-PVP was set to 10 ng/mL per the manufacturer's recommendations. MDPV has over 90% cross-reactivity per the kit insert. Precision at 50% below the decision point (low – 5 ng/mL), at the decision point (cutoff – 10 ng/mL), 50% above the decision point (1.5X – 15 ng/mL), and 100% above the decision point (high – 20 ng/mL) was monitored in triplicate over 5 days for α-PVP and MDPV. Thirty previously analyzed samples were evaluated to determine false positive/negative rates and assess the assay's ability to reliably detect compounds at concentrations commonly observed in routine casework. Interference was evaluated in ante- and post-mortem blood specimens and commonly encountered stimulants including amphetamine, methamphetamine, and phentermine. As a component of their in-house validation, Randox studied the following parameters: intra-assay precision, limit of detection, interference, and cross-reactivity.

Result: Intra-day precision (CVs) was 2.0% – 13.6% for alpha-PVP and 2.8% - 16.3% for MDPV. Between-day precision is shown below. All concentration points had CVs of 15% or less.

	Between-day Precision (CV%)			
Target	Low (5 ng/mL)	Cutoff (10 ng/mL)	1.5X (15 ng/mL)	High (20 ng/mL)
Alpha-PVP	9%	10%	13%	12%
MDPV	13%	14%	15%	14%

There were no false negative and false positives in the 30 previously analyzed cases including six known positive cases. There was no interference in negative blood specimens or with commonly encountered drugs amphetamine, methamphetamine, or phentermine spiked at 500 ng/mL.

Conclusion/Discussion: We are the first forensic toxicology laboratory to validate the Randox α-PVP/MDPV ELISA assay. Sufficient precision around the cutoff and the lack of false positives/negatives indicate that the assay has the ability correctly identify a sample as presumptively positive or negative. Laboratories should strive to their increase scope of analysis and services provided to their customers. We offer a highly specific preliminary test for two of the most prominent synthetic cathinones, α-PVP and MDPV.

Keywords: α-PVP, MDPV, ELISA

P53
Validation of Immunoassays for the Screening of Dried Blood Spots (DBS)

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

Background/Introduction: Based on recommended validation parameters set forth by the manufacturer, immunoassay screening parameters were fully validated for drugs in dried blood spot specimens at recommended whole blood cutoffs.

Objective: To validate Immunalysis ELISA (enzyme-linked immunosorbent assay) kits for drugs in dried blood spots (DBS) and to identify any limitations during the course of validation.

Method: All kits were tested for intraday precision and accuracy by calculating the grand mean % B/B° (% sample bound / maximum binding), standard deviation (SD) and coefficient of variation (CV%) of three independent runs with 6 replicates per run (n=18). The calculated 2X SD was applied to the grand mean and % B/B° was determined. Acceptance criteria for each validation included CV ≤20% and % B/B° range for one calibration level must not overlap with another calibration level. All immunoassays were fully validated at 50% (low cal), 100% (cut-off cal), and 200% (high cal) of the recommended cutoffs on a TECAN Genesis RSP 200 (Mannedorf, Switzerland). Sample volumes were adjusted to give the best possible separation with the most difficult aspect being adequate distinction between 100% and 200% concentrations. All controls were prepared by fortifying negative blood with analyte concentrations at 50, 100, and 200% the recommended cutoffs. For each DBS 30µL of each control was then spotted on to a Whatman (Cardiff, UK) 903 collection paper card.[1] The cards were allowed to dry overnight at room temperature and were punched out the following day into 12x75mm test tubes. To simulate a 1:10 dilution, 270µL of 100mM phosphate buffer (pH 7.0) containing 0.1% w/v bovine serum albumin (BSA) was added to each tube. Samples were mixed for five minutes and allowed to soak for an additional 10 minutes. Liquid was removed and plated at optimal sample volumes listed below. ELISA protocol was followed based on kit inserts. DBS extraction efficiencies were determined by LC-MS/MS. DBS were prepared and extracted following the same protocol for ELISA. Samples where then extracted by previously validated whole blood methods and compared against whole blood curves. All extraction efficiencies were between 90 – 105% with THC-COOH being the lowest at 90%.

Result: The results are shown in the table.

				% B/B° range		
Drug	**Cut-off (ng/mL)**	**CV (%)**	**Sample volume (µL)**	**Negative – Low cal**	**Low cal – Cutoff**	**Cutoff – High cal**
THC-COOH	10	< 5	25	94.903 – 79.176	78.781 – 69.429	57.285 – 49.921
Amphetamine	20	< 3	50	47.571 – 45.874	37.324 – 35.940	27.373 – 24.457
Methamphetamine	20	< 3	50	58.588 – 54.074	51.071 – 45.794	40.552 – 36.916
Benzoylecgonine	25	< 4	10	84.326 – 82.144	79.045 – 72.821	66.373 – 57.668
Oxazepam	20	< 3	40	79.009 – 72.629	63.706 – 60.914	47.637 – 43.131
Morphine	25	< 2	10	56.931 – 54.825	42.961 – 40.174	33.117 – 31.505
Oxycodone	25	< 7	10	27.193 – 22.467	16.983 – 12.801	10.101 – 9.187

Conclusion/Discussion: Immunoassay screening parameters for the analysis of drugs in dried blood spots was successfully validated according to the manufacturer guidelines at the recommended cutoff concentrations. Limitations were predominantly associated decreased sample volume available for testing.

References: 1. Bynum, N., Moore, K., Grabenauer, M. (2015) The Importance of Evaluating Internal Standard Addition Methods in Dried Blood Spot Analysis. SOFT.

Keywords: Dried Blood Spots, ELISA, Validation Guidelines

P54

Fentanyl Data in Fatalities and Impairment (DUID) Cases: A 5-year (2010-2016) Retrospective Study

Carrie D. Mazzola*, Eric S. Lavins, Hugh B. Shannon, Rindi N. Rico, Claire K. Kaspar, Paul D. Boggs, Harold E. Schueler, Thomas P. Gilson; Cuyahoga County Regional Forensic Science Laboratory, Toxicology and Drug Chemistry Department, Cuyahoga County Medical Examiner's Office, Cleveland, OH

Background/Introduction: The Toxicology Department at the Cuyahoga County Medical Examiner's Office (CCMEO) and the Cuyahoga County Regional Forensic Science Laboratory, has observed an epidemic of fatal fentanyl deaths and an increase in Driving Under the Influence of Drugs (DUID) cases over the past 5 years (2010-2016 first quarter). Fentanyl, N-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl]propanamide, is a potent, synthetic opioid with rapid onset and short duration of action. It is a strong mu opioid receptor agonist and 100 times more potent than morphine. Clinically used as an adjunct to surgical anesthetic and breakthrough pain, it is also produced in clandestine laboratories in powder and pill form. This has caused a resurgence of illicit use of fentanyl and its analogues. Data generated from postmortem cases received at the CCMEO over the past five years indicate there were 13 fentanyl related deaths in Cuyahoga County in 2010 (1.02 deaths per 100,000 people), in contrast to 77 fentanyl related deaths in the first quarter of 2016 (24.25 deaths per 100,000 people). Fentanyl related DUID cases have increased 100% since 2010. Interestingly, many of the recent DUID fentanyl blood concentrations fell into ranges generally recognized as toxic or fatal.

Objective: To present the demographics, results, incidence, and frequency of fentanyl deaths, including polysubstance abuse in postmortem, DUID and drug chemistry cases from 2010 to 2016 in Cleveland, Ohio (population ~396,815) and Cuyahoga County as a whole (population ~1,280,122).

Method: In the 5-year period analyzed, there were 14,773 autopsies or death investigations performed at CCMEO with full toxicology requested on 9,932 cases and 1,832 DUID submissions. The CCMEO toxicology and drug chemistry departments performed standard comprehensive testing on multiple specimens and submissions using gas chromatography/mass spectrometry. Testing was performed, in the same manner, for DUID cases as requested by individual agencies. Fentanyl methodology and sensitivity levels were consistent throughout the study period with an LOD of 0.5 ng/mL and an LRL of 1.0 ng/mL.

Result: There has been a remarkable epidemiological change in the number of fentanyl related deaths and usage in Cuyahoga County, including Cleveland, Ohio and its suburbs. Postmortem fentanyl demographics are white (80%) males (75%) with ages evenly distributed among 19-60 years. Usage is equally dispersed between individuals in urban Cleveland and its suburbs. In 2010, only 4.86% of poisoning deaths were due to fentanyl related intoxications. In 2014, this increased to 10.30% due to fentanyl related intoxications and dramatically increased in 2015 to 24.86%. Fatal fentanyl concentrations ranged from 1.0 to 80.0 ng/mL. Approximately, 20% of fentanyl fatalities were due to fentanyl alone. The majority were a result of polysubstance intoxications; primarily heroin/fentanyl, cocaine/fentanyl, ethanol/fentanyl, benzodiazepine/fentanyl, or a combination thereof. Similarly, fentanyl was pertinent in only 0.47% of the DUID cases in 2010 compared to 3.88% of cases in 2015 and 4.47% of cases in the first quarter of 2016. Of the 41 total DUID cases positive for fentanyl, 7 were positive for fentanyl alone with concentrations ranging from 5.0 - 25.2 ng/mL with a median of 8.5 ng/mL and a mean of 10.2 ng/mL. A 25.2 ng/mL DUID fentanyl was reported in a 2015 case from a Cleveland suburb. In the remaining 34 DUID cases, fentanyl was present in conjunction with other substances. The fentanyl blood concentrations range from <1.0 - 11.0 ng/mL with a median of 4.1 ng/mL and mean of 4.8 ng/mL. Fentanyl was reported as positive for urine DUID cases. Drug chemistry fentanyl positive submissions increased from 0.01% in 2010 to 4.3% of positive submissions, for the first quarter of 2016.

Conclusion/Discussion: This study demonstrates that there has been an increase in fentanyl related deaths and fentanyl related DUIDs in Cuyahoga County and surrounding counties. The increase in fentanyl follows the statistical increase of heroin abuse and is due to low cost and high availability of the clandestinely manufactured, illicit distribution of a non-traditional formulation of fentanyl. Fentanyl is also being sold fraudulently as heroin, oxycodone, benzodiazepine tablets, or compounded with other drugs to unsuspecting users.

Keywords: Fentanyl, DUID, Medical Examiner

P55
Postmortem Distribution of Tenocyclidine

Meghan Melnyk*, Rebecca A. Jufer Phipps, Barry S. Levine, Melissa Brassell, David Fowler; Office of the Chief Medical Examiner (OCME), State of Maryland, Baltimore, MD

Background/Introduction: Tenocyclidine (1-[(2-thienyl)cyclohexyl]-piperidine) is a drug of abuse which is relatively unseen in the United States. An analog of phencyclidine (PCP), Tenocyclidine (TCP) is classified as a dissociative anesthetic with both stimulant and hallucinogenic potential. TCP is capable of easily crossing the blood brain barrier. Animal studies have estimated TCP to be more potent than its analog PCP. TCP is currently considered a schedule one drug under the U.S. Controlled Substances Act. It is fairly inexpensive to acquire and can be taken orally.

Objective: Analysis of biological specimens for TCP allows for the study of its postmortem distribution. Due to the limited case studies and information available, this data will provide medical examiners and toxicologists with an analytical method and postmortem concentrations of TCP to use as a reference when needed.

Method: The OCME of the State of Maryland has investigated one death related to TCP use.
Case History: A 35 year old African American male was found unresponsive on his living room floor. He was last known alive by his mother the previous night around midnight. Since the individual has a past history of sleeping throughout the day and into the evening, the mother periodically attempted to wake the subject without success as the day progressed. Emergency services were eventually called and the subject was pronounced at the scene. The decedent's room contained a vial of suspected PCP. It was reported that the decedent had a history of drug abuse, specifically PCP and synthetic marijuana, since age sixteen. Beyond an enlarged heart and marked pulmonary edema, autopsy results were unremarkable. Comprehensive toxicology testing was performed including volatiles, an acidic neutral drug screen, an alkaline drug screen and ELISA for morphine, benzodiazepines and oxymorphone. The case was positive for PCP and TCP but negative for other drugs.
TCP was identified in an alkaline drug screen, which involved an alkaline extraction of specimens followed by detection with gas chromatography - nitrogen-phosphorous detection (GC-NPD) and confirmation by gas chromatography - mass spectrometry (GC-MS). TCP elutes shortly before PCP on an HP-5 column and prominent GC-MS ions are 97, 165, 206, and 249. A quantitation method was developed and validated for TCP. All matrices were extracted by the same procedure, except bile (3:5) and liver/kidney (1:5), which were extracted at a dilution. Briefly, internal standard (Mepivacaine) was added to specimens which were alkalinized and extracted with n-butyl chloride then back extracted into sulfuric acid and finally alkalinized and extracted into methylene chloride. Isopropanol was added and the extract was evaporated to the isopropanol layer which was injected into the GC-NPD for analysis. The method was linear from 0.05 mg/L to 2.0 mg/L. A six-point calibration curve and two control concentrations were used for quantitation. An administrative reporting limit of 0.05 mg/L was used.

Result: Case specimen concentrations of TCP and PCP (mg/L or mg/kg) are summarized below.

Analyte	Heart Blood	Liver	Kidney	Urine	Bile	Vitreous Humor
TCP	0.29	0.48	0.22	0.45	Positive	0.08
PCP	0.61	0.82	0.39	0.80	Positive	0.14

Conclusion/Discussion: TCP was detected in all specimens analyzed, including vitreous humor, making it a suitable specimen to indicate recent use of TCP in instances when blood is unavailable. Urine also contained a higher concentration of TCP relative to blood, making it a suitable specimen to screen for TCP use. Distribution of TCP appears similar to that of PCP, which is consistent with their structural similarities. Since TCP is reported to be more potent then PCP with similar effects, it is likely that the TCP concentrations in the reported case are in the toxic range. However, very little information is currently available due to the absence of postmortem TCP data in the literature.

Keywords: Tenocyclidine, PCP, Postmortem

P56

Prevalence of Recreational Drugs and New Psychoactive Substances in a Cohort of Patients Presenting to an Urban Emergency Department (ED) with Suspected Acute Recreational Drug Toxicity

Michelle Wood*[1], Rachelle Abouchedid[2], David M. Wood[2], Nayan S. Mistry[1], John R.H. Archer[2], James Ho[2], Paul I. Dargan[2]; [1]Waters Corporation, Wilmslow, UK, [2]Guys and St Thomas' NHS Foundation Trust, Clinical Toxicology, London, UK

Background/Introduction: The Crime Survey for England and Wales (CSEW) is published by the Office for National Statistics and includes measurement of the extent, and trends, in illicit drug use amongst adults aged 16-59 years. Key findings from the 2014/15 report indicated that around 8.6% of adults had taken an illicit drug in the last year[1]. The figure for young adults (16-24 years) over the same period, was more than double at ~19.4%. The survey also revealed that around 1 in 40 of young adults took a new psychoactive substance (NPS) in the last year and that NPS drug use was also more common amongst individuals who frequent pubs and nightclubs. Recreational drugs and NPS are common reasons for presentation to the ED with acute toxicity however, there is limited data on the actual drugs responsible for the presentations and self-reported drug use used may be unreliable.

Objective: The aim of this study was to assess the drugs present in a cohort of patients with suspected acute recreational drug/NPS toxicity.

Method: Samples were collected over a period of 6 months, from a cohort of patients who had presented to the Emergency Department of a central London Hospital with acute recreational drug toxicity and had blood samples taken according to routine clinical care. Surplus plasma samples were anonymised and sent to the laboratory for comprehensive drug screening. Briefly, samples were prepared by liquid/liquid extraction and screened using UPLC in combination with time-of-flight (TOF) mass spectrometry. Chromatographic separation was achieved using a Waters ACQUITY UPLC® I-Class system fitted with a HSS C_{18} column and eluted with a mixture of ammonium formate at pH 3 and acetonitrile containing formic acid. Data were acquired using a Waters Xevo G2 XS QTOF in MS^E mode and thereafter processed using the UNIFI™ Toxicology Screening Solution (Waters) which comprised comparison to a database containing more than 1400 drugs and metabolites. Identification of substances by UPLC-TOF-MS^E was based on a combination of retention time and a mass 'fingerprint' for each analyte, the latter comprising the accurate mass of the precursor ion and fragment ions.

Result: One-hundred and ninety-one samples were available for characterisation by UPLC-TOF-MS^E. Of these, 83.8% screened positive for at least one illicit drug substance. The most common drug detected was mephedrone and its metabolite, which was detected in 65 samples (40.6% of samples containing illicit drug substances). The most commonly detected drug class however was the amphetamines, which were present in 54% of positive cases (methamphetamine, amphetamine, MDMA). Cathinones (including methylone, ethylone and butylone) represented 45.5% of illicit-positives, followed by cocaine (38%), opiates (26%) and ketamine (6.9%). In 45% of cases, only a single illicit drug class was detected, however more commonly two or more drug classes were detected. The most commonly detected illicit class combination was cathinone/amphetamine (27% of all samples), followed by cocaine/opiates (10%); cocaine/amphetamines (9.3%) and cocaine/cathinones (6.9%). Other notable detections included ketamine, ethylphenidate, methiopropamine and alpha-PVP. Benzodiazepines were also found in 48.1% of samples; in many cases these are likely to be associated with therapeutic use in the ED.

Conclusion/Discussion: UPLC-TOF-MS^E was used for its ability to screen for both established recreational drugs (targeted analysis) in addition to novel psychoactive substances (non-targeted analysis). In this study the amphetamines were the most commonly detected drug class followed by the cathinones. Mephedrone was the most commonly detected illicit drug substance. The majority of the mephedrone-containing samples were also positive for other recreational drugs. Studies such as this, with broad-based, toxicology screening, are important to determine the actual drugs used by individuals presenting to hospital with acute recreational drug toxicity and to determine trends in the use of, and toxicity associated with, novel psychoactive substances.

References:[1]Home Office for National Statistics (2015) Drug Misuse: Findings from the 2014/15 Crime Survey for England and Wales – second edition. Accessed online (April 12, 2016)

Keywords: NPS, TOF, Screening

P57
Automated Drugs of Abuse Analysis from Oral Fluid

Shahana Huq*, Seyed Sadjadi, Laura Snow, Sean Orlowicz; Phenomenex Inc., Torrance, CA

Background/Introduction: Drug testing from oral fluid specimens has been gaining popularity over other sample types such as urine and whole blood. The most likely reasons for this popularity are low chance of sample adulteration and cheating, easy and non-invasive nature of sample collection. Not surprisingly, there are many oral fluid collection (OFC) devices to address the rising need. Many OFC devices are available with collection applicator and preservative (or extraction) solution to help extend the stability of analytes. In this work, we present an automated SPE procedure to extract a large variety of compounds from two different OFC devices with good recovery and reproducibility.

Objective: The objectives of work were to set up an automated SPE method that will successfully extract compounds of varying chemical properties from different OFC devices.

Method: We chose a group of analytes present in most common pain panel. The group represents acidic (COOH-THC, phenobarbital), basic (amphetamine, hydrocodone, methadone), zwitter ionic (Gabapentin, pregabalin), neutral (carisoprodol, meprobamate), hydrophilic (oxymorphone, morphine) and hydrophobic (lorazepam, temazepam) compounds. In all, there were fifty (50) analytes in our compound panel.
The LC/MS/MS method utilized a Kinetex Biphenyl 2.6 um, 50x3.0 mm column with a simple 0.1% formic acid in water and methanol as mobile phase. The same column and MP were used for the analysis of both acidic and basic compounds. A fast LC gradient resulted in total run time of 5 min. The detection were carried out on a SCIEX API 5000 equipped with ESI source. For basic compounds the MS was operated under positive polarity and in a separate injection, the acidic compounds were analyzed in negative polarity.
Two separate SPE cartridges are used: 30 mg/3 mL Strata-X-A for acidic analytes and 30 mg/3 mL Strata-X-C for basic compounds. Samples are composed of 1:2 and 1:3 (depending on the device) spiked saliva:OFC device preservative buffer. A Tecan Freedom Evo 100 automated sample handling system carried out the various steps in sample dilution and extraction. Majority of the surfactants in OFC device buffers were removed with 50% aqueous acetone wash and we used this solvent as strong wash to clean up the SPE sorbent bed

Result: Calibration curves were constructed from spiked saliva revealed a range from as low as 0.25 ng/mL to as high as 300 ng/mL for compounds of interest. Several analytes (methadone, diazepam) displayed non-linear calibration curve. For these analytes a quadratic fit with 1/x weighting factor seemed more appropriate. Two levels of QC samples were analyzed producing a precision range of 3-15% and accuracy of 87-115% from target spike (n = 4).

Conclusion/Discussion: The automated extraction procedure worked well for the two OFC devices. All ionizabe analytes showed adequate recoveries. However, neutral compounds (carisoprodol and meprobamate) were lost during the extraction.

Keywords: Oral Fluid, SPE, Automation

P58

LC-MS/MS Method Development Challenges for the Separation of 43 Opioids and Metabolites

Justin Steimling*, Sharon Lupo, Frances Carroll; Restek Corporation, Bellefonte, PA

Background/Introduction: The use of liquid chromatography coupled with mass spectrometry (LC-MS/MS) has become a routine method of analysis in therapeutic drug monitoring and clinical toxicology labs. LC-MS/MS provides sensitivity, speed, and specificity when analyzing drugs in complex biological matrices, such as plasma, serum, and urine. Because of the similarity of many opioids and metabolites, chromatographic separation becomes increasingly difficult due to the number of structural isomers and the need to chromatographically resolve these isomers due to their identical mass spectral fragmentation patterns.

Objective: The intent of this study was to develop a comprehensive method for 43 common opioids and their metabolites on a Raptor™ Biphenyl column.

Method: Forty-three opioids and metabolites were prepared in three stock solutions. Compounds of the same molecular weight were distributed into different solutions to facilitate compound optimization and identification. Following compound optimization by infusion, the stock solutions were diluted in water and injected into a Shimadzu Nexera UHPLC equipped with a Raptor™ Biphenyl 2.7 µm 100x2.1mm column equipped with EXP® 2.7µm, 5 x 2.1mm guard column. Detection was performed using an AB SCIEX API 4500™ MS/MS with electrospray ionization in positive ion mode and scheduled multiple reaction monitoring (MRM). Scouting gradients were used to investigate the effects of mobile phase composition on opioid retention and peak shape.

Result: Each solution was analyzed using a linear scouting gradient program and water and methanol mobile phases varying modifiers, acidic (0.1% formic acid), neutral (5 mM ammonium acetate), and buffered acidic (0.1% formic acid and 5 mM ammonium formate). It was determined that the neutral mobile phase was the only mobile phase which could achieve resolution of all structural isomers. However, when compared to the acidic mobile phase, the neutral mobile phase displayed peak broadening and tailing. The buffered, acidic mobile phase exhibited acceptable peak shape but a distinct decrease in response for buprenorphine and norbuprenorphine was apparent when compared to observed responses for the acidic mobile phase. The acidic mobile phase provided the best overall sensitivity and peak shape for the opioids. All structural isomers could be resolved using this mobile phase with the exception of dihydrocodeine and noroxycodone. Due to the polarizable nature of the Raptor™ Biphenyl phase, analyte resolution can be influenced by the choice of protic versus aprotic organic solvents. When the organic solvent of the acidic mobile phase was switched from methanol to acetonitrile complete resolution of dihydrocodeine and noroxycodone was achieved while maintaining resolution for all other isomers.

Conclusion/Discussion: Resolution of all structural isomers and retention of early-eluting analytes was achieved using the optimized mobile phases and instrument conditions listed in Table 1. The final optimized separation utilizes a Restek Raptor™ Biphenyl 2.7µm, 100 x 2.1mm column equipped with a Raptor™ Biphenyl EXP® 2.7µm, 5 x 2.1mm guard column. The gradient run time is 3.5 minutes, with a total cycle time of 5.5 minutes.

Keywords: LC-MS/MS, Opioids, Metabolites

P59

Detection and Semi-Quantitative Determination of Designer Benzodiazepines in Serum Using LC-MS^n

Ronja Peter[1,2], Aurore Wicht[2,3], Björn Moosmann[2], **Jürgen Kempf*[2]**; [1]Offenburg University of Applied Sciences, Offenburg, Germany, [2]Institute of Forensic Medicine, Medical Center – University of Freiburg, Faculty of Medicine, University of Freiburg, Germany, [3]Ecole des Sciences Criminelles, University of Lausanne, Lausanne, Switzerland

Background/Introduction: In 2012 the group of New Psychoactive Substances (NPS) including numerous synthetic cannabinoids and designer stimulants ("bath salts") was extended by benzodiazepine-type compounds. At first, benzodiazepines like phenazepam and etizolam - which are still prescribed in some countries - were sold on the internet as recreational drugs. In the last years, the group of so-called designer benzodiazepines was enlarged by compounds that either are precursors (e.g. diclazepam) or active metabolites (e.g. norfludiazepam) of known benzodiazepines or combine structural properties of different classical benzodiazepines (e.g. flubromazolam). Considering the fact that patents and scientific literature describe the synthesis and detailed results of animal model studies for more than a hundred different benzodiazepines, it can be assumed that this sub-group of NPS will extend quickly in the future.

Objective: The aim of this project was to develop an easy-to-use screening-method to detect and identify prescription and designer benzodiazepines and additionally obtain semi-quantitative information in a single run.

Method: Serum sample preparation was performed according to an established liquid-liquid extraction (LLE) protocol. For chromatographic separation, a 13-minute LC gradient using formic acid/acetonitrile and a Dionex Acclaim RLSC C18 100 x 2 mm column were used. An amaZon speed ion trap MS (Toxtyper®, Bruker Daltonik) operating in ESI positive mode was used to generate MS^2 and MS^3 spectra according to a scheduled precursor list (SPL) triggered acquisition process. Compounds were identified by an in-house generated spectral library, containing retention time, MS and MS^2/MS^3 information of currently 53 benzodiazepines. To obtain semi-quantitative results, a one-point calibration was used. The linear range of the method was evaluated by fortifying blank human serum with benzodiazepine standards from 5 to 500 ng/ml. A 50 ng/ml calibrator was chosen for one-point calibration.

Result: The current spectral library contains 12 designer benzodiazepines and those prescription benzodiazepines most common in Germany. The method can easily be extended once new designer benzodiazepines emerge on the drug market or according to specific needs of the user.
The limit of detection was 5 ng/ml for the majority of the analytes, whereas six compounds could only be detected at concentrations above 10 ng/ml. Nifoxipam, known to have a poor ESI response and being highly instable in serum, was the only compound that could not be detected at practically relevant concentrations in serum. For each analyte a linear calibration range (cal_{Low} to cal_{High}) was determined and calculated concentrations within this range are reported as semi-quantitative result in the automatically generated report. Findings below or above the linear range are reported as '$<cal_{Low}$' or '$>cal_{High}$', respectively. Semi-quantitative results were found to vary between ± 20 and ± 40 % at the lower end of the calibration range and ± 10 to ± 25 % at medium and high concentrations.

Conclusion/Discussion: The presented method allows automated identification and semi-quantitative determination of 53 benzodiazepines, including 11 designer benzodiazepines. Limits of detection of the assay allow the detection of sub-therapeutic concentrations or concentrations in the low therapeutic range for the majority of medical benzodiazepines, making the screening applicable for clinical and forensic analysis. Semi-quantitative analysis enables a quick toxicological evaluation of the results and helps to decide on the analytical strategy in case work with limited sample volume available. Although this approach requires a more time consuming sample preparation when compared to routine immunoassays, unambiguous identification and semi-quantitative determination of compounds also offers more detailed information.

Keywords: Designer Benzodiazepines, Semi-Quantitative Screening, LC-MS^n

P60

Analysis of U-47700 in Urine by Liquid Chromatography-Tandem Mass Spectrometry

Steven W. Fleming*[1,2], A.M. Spiekerman[2]; [1]Kentucky Toxicology Consulting LLC, Whitesburg, KY, [2]Gulfstream Diagnostics, Dallas, TX

Background/Introduction: U-47700 (3,4-dichloro-N-[2-(dimethylamino)cyclohexyl]-N-methylbenzamide) is a synthetic opioid developed in the 1970s by investigators at The Upjohn Company. A study of U-47700 in guinea pig brain demonstrated binding at the μ-receptor. U-47700 has not been studied in humans; however, typical opioid pharmacology is expected. As abuse of research chemicals increase, U-47700 may be abused as a substitute for other opioids. This year there are reports of its abuse in Texas, Florida, Belgium, and Sweden. The drug is illegal in Sweden and Finland but not scheduled in the United States.

Objective: To develop a method for the quantitation of U-47700 in urine specimens.

Method: Blank human urine (UTAK Laboratories) samples were spiked with U-47700 (Cayman Chemical). A reference standard for metabolites is not commercially available, but the authors suggest urinary metabolites to include N-demethylated and hydroxylated compounds. The urine sample (0.25mL) was treated with internal standard (Norpropoxyphene-D5), beta-glucuronidase, 0.1M sodium acetate buffer, and extracted using SPEware PSCX cartridges. The eluents were evaporated to dryness, and reconstituted with methanol and aqueous mobile phase A (5mM ammonium acetate and 0.01% formic acid). The extracts were subsequently analyzed by positive electrospray ionization liquid chromatography tandem mass spectrometry (LC/MS/MS) using the following transitions (329.1-> 284.0 and 329.1 -> 172.9) for U-47700. Injections (10μL) were separated within 8 minutes using a gradient profile of aqueous mobile phase A and mobile phase B (0.01% formic acid in methanol) with a 0.5 mL/min flow rate on an Agilent Poroshell 120 EC-C18 (2.1mm x 100 mm, 2.7μm) column that was maintained at 50°C. Approximately 70 other drugs (including benzodiazepines, opiates, opioids, and synthetic cannabinoids/cathinones) were spiked into mobile phase and analyzed to determine if they interfered with U-47700.

Result: The limit of detection and quantitation was determined to be 0.05 ng/mL. The method exhibited a linear calibration range from 0.05 - 1250 ng/mL with a weighting of $1/x^2$ ($r2 > 0.995$). However, the cutoff of the assay was administratively set to 5 ng/mL. The calibration model was determined by analyzing fifteen concentrations (0.05 – 5000 ng/mL) over three days. A mean (n=3) within 10% of the target concentration was considered acceptable for establishing the calibration range. Interday and intraday precision studies (n=11) were completed at three concentrations (1, 7.5, and 500 ng/mL) with interday %Coefficient of Variations of 5%, 1%, and 3% respectively. Interday and intraday accuracy studies (n=11) were completed at the same concentrations with interday % accuracy of 110%, 103%, and 98% respectively. Extraction efficiencies and matrix effects were 43-66% and -21 to -3%, respectively. U-47700 was stable (±20% of target) after 72 hours at 6°C (in the autosampler), 16 hours at room temperature (21-27°C), 72 hours at 4°C and three freeze-thaw cycles. Also, the suspected interferants analyzed did not interfere with U-47700. Daily LC/MS/MS acceptance criteria included a six point calibration curve with a correlation coefficient of 0.99 or greater, retention time ±2.5% with ion ratios ±20% of calibrators, and QC results ±20% of the target concentration.

Conclusion/Discussion: Since the validation of this methodology, U-47700 has been detected in three urine specimens. SPE followed by LC/MS/MS analysis was determined to be a sensitive and specific method for the quantitation of U-47700 in urine. Detection of this potent research chemical is imperative to the forensic and clinical toxicology community as typical targeted analysis will not detect this analyte.

Keywords: Synthetic Opioid, U-47700, Designer Drug

P61
Disposition of the Synthetic Cannabinoid CP47,497 in the Mouse

Carrol R. Nanco*[1], Kim Samano[2], Justin L. Poklis[2], Carl Wolf[1,3], Alphonse Poklis[1, 2, 3]; [1]Forensic Toxicology Laboratory, Medical College of Virginia Hospitals and Physicians of Virginia Commonwealth University Health Systems, Departments of [2]Phamacology & Toxicology and [3]Pathology, Virginia Commonwealth University, Richmond, VA

Background/Introduction: CP47,497, (2-[(1R,3S)-3-hydroxycyclohexyl]- 5-(2-methyloctan-2-yl)phenol), a cyclohexylphenol ("CP") prototypical synthetic cannabinoid, is one of the designer drugs found in herbal incense products (HIPs). It has similar psychotropic effects as marijuana but has ten times higher binding affinity to the CB1 receptor in in-vitro studies. Initially synthesized in the 1980s as a pharmacological tool to help elucidate the structure of the cannabinoid receptor(s) in brain and peripheral tissues, it along with other HIPs have emerged as significant public health and safety concerns due to their marijuana-like high effects produced when the product is smoked. Toxicity symptoms reported include anxiety, paranoia, tachycardia, irritability, hallucination, numbness, seizures, high blood pressure, drowsiness, and slurred speech. As of July 21, 2012 widely abused synthetic cannabinoids including CP47,497 were listed as Schedule 1 drugs under the Synthetic Drug Abuse Prevention Act by the US Drug Enforcement Agency. HIPs are manufactured in clandestine laboratories without quality control, therefore CP47,497 may still be found in products labeled and sold as 'not for human consumption'. The authors are not aware of any published studies on the disposition of CP47,497 in the mouse.

Objective: To determine the disposition of the prototypical synthetic cannabinoid, CP47,497 in mouse tissue after intraperitoneal (*i.p.)* injections of 30mg/kg of body mass.

Experimental Method: Drug naïve male mice (25–35g) (n=10) received *i.p.* injections of either vehicle (n=4) (ethanol:emulphor:saline in a ratio of 1:1:18) or 30mg/kg CP47,497 (n=6). One hour post-injection, mice were sacrificed and blood and tissue specimens collected. Blood, brain, liver, spleen, kidney, and heart tissue were stored at -80C until analysis. Experiments were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals.

Analytical Method: A Waters Acquity™ UPLC system with a TQD was used for the analysis of CP47,497 in mouse blood, brain, heart, kidney, liver and spleen. Matrix matched calibrators and controls were analyzed with each tissue set. Drug-free mouse tissue homogenates were fortified at 50-2000ng/g except liver which was fortified at 100-5000ng/g. Homogenates were prepared for brain, heart, kidney, and spleen tissues at 1:4 dilution with water and a 1:10 dilution for liver. 500 ng/g of CP47,497-d_{11} was used as the Internal standard. Calibrators, controls and samples were extracted with acetonitrile.

Result: One hour post *i.p*. administration of CP47,497 at 30mg/kg, tissue concentration ranges were: Blood 0.198-0.828mg/L (mean 0.46mg/L); Brain 1.66-1.89 mg/kg (mean 1.88mg/kg); Heart 2.31-10.1mg/kg (mean 7.49mg/kg); Kidney 16.9-61.9mg/kg (mean 29.5 mg/kg); Liver 25.7-60.3mg/kg (mean 44.6mg/kg) and Spleen 3.23-36.7mg/kg (mean 20.4mg/kg). The range of mean concentration ratios of blood to tissue in increasing order were: brain/blood = 4.0; heart/blood = 16; spleen/blood = 43; kidney/blood = 63 and liver/blood = 97. This increasing order of blood to tissue ratios of C47,497, while of greater magnitude, are of the same order observed by *Brunet et al. (Forens. Sci. Intern. 161:169, 2006*) following *i.p.* injection of THC in the large white pig; lowest ratio blood to brain and highest ratio blood to liver. CP47,497 was not detected in vehicle-treated mice in the study.

Conclusion/Discussion: Tissue concentrations of CP47,497, one hour post *i.p.* administration of 30mg/kg, were significantly higher than blood concentrations. A low concentration of CP47,497 in blood compared to the higher concentrations in all the tissues indicate a high apparent volume of distribution. This is apparently due to the highly lipophilic nature of CP47,497. The presented data indicate that CP47,497 should be easily identified in tissues one hour post administration.

Acknowledgements: This project was supported by the National Institute on Drug Abuse (NIDA) Center for Drug Abuse grants P01DA009789, F31DA033183 and P30DA033934.

Keywords: CP47,497, Synthetic Cannabinoid, Tissue Distribution

P62

Improved Detection of Cathinones, 2C Amines and 25NBOMe Designer Drugs in Urine by Derivatization and Gas Chromatography Mass Spectrometry (GC-MS)

Milad Webb*[1], Gregory Janis[2], Hema Ketha[1]; [1] Department of Pathology, University of Michigan Hospital and Health Systems, Ann Arbor, [2]Medtox Laboratories, Laboratory Corporation of America, St. Paul, MN

Background/Introduction: The detection of cathinones, synthetic phenethylamines (2C-amines, amphetamine derivatives) and NBOMe compounds is vital to provide appropriate care for overdosed or exposed patients. However, the growing repertoire and rapid evolution of "legal highs" pose significant clinical and technical challenges to clinical toxicology laboratories and clinicians. High resolution instrumentation remains generally unavailable in hospital settings, and available techniques such as immunoassay (IA) and GC-MS screening suffer from poor detection limits for these compounds.

Objective: We set out to develop an improved qualitative GC-MS method identifying 2C-amines, cathinones and NBOMe drugs. Through a derivatization procedure (DER-GCMS), our primary goal was to improve the limit of detection (LOD) of the targeted analytes compared to routine urine GC-MS drug screen (UD-GCMS).

Method: Seventeen test solutions were prepared (2CB, 2CC, 2CD, 2CE, 2CI, DOI, escaline, 25C-NBOMe, 25B-NBOMe, 25I-NBOMe, bromo-dragonFLY, 2CB-FLY, MDPV, methylone, butylone, MDMA and mephedrone) in drug free urine (UTAK Laboratories) using certified reference solutions (Cerrilliant® Corporation). Assay performance at LOD and the qualitative identification of targets were compared between the two analytical techniques described below. LOD determination (signal-to-noise (s/n)>2000) compounds were identified in urine solutions (50ng/mL) manually by comparing extracted-ion chromatograms of expected m/z established from reference solutions. Both procedures were additionally exercised on samples from the ED with suspected designer drug use.

1. UD-GCMS: 1 mL urine samples were mixed with 50µL of dioctylphthalate as an internal standard, 5mL of pH=10.5 phosphate buffer and extracted with ethyl acetate(EA)/isopropyl alcohol (90/10) for 15 min. The organic layer was separated by centrifugation, transferred and dried under N_2. The residue was redissolved in 100µL EA and 0.5µL was injected and analyzed on a GC-EI-MS (Agilent Model GC-7890B & MS-5977) equipped with a J&W Ultra 2, 12m, 0.2mm i.d., 0.3µm film column.
2. DER-GCMS: 1 mL urine sample were mixed with 50µL of dioctylphthalate, 1mL of 5M NaOH and were extracted via vortex mixing with 1mL of hexane. The organic extract was separated via centrifugation and derivatized with 4-5 drops of penta-fluoropropionic acid anhydride (PFPA) incubated at 45°C for 15-20min. The contents were dried under N_2 at 45-50°C and analyzed as above.

Result: The LOD for 16 compounds (except MDPV) on the UD-GCMS method was 500ng/mL and improved to 50ng/mL using the derivatization procedure. As a tertiary amine, the LOD of MDPV was comparable (50 ng/mL) by both methods. An approximately 40 fold s/n gain was achieved by DER-GCMS. Poor s/n was observed for 2C-(primary) amines by the UD-GCMS method likely because of the higher basicity and volatility of this class of stimulants. The higher extraction pH and derivatization by DES-GCMS improved the detection of 2C-amines. 2CB was detected in a patient sample using DES-GCMS with a 45 fold higher s/n compared to the UD-GCMS procedure. The sample was subsequently confirmed by LC-TOF at an outside reference laboratory.

Conclusion/Discussion: Extracting at a higher pH and derivatizing with PFPA improved the assay LOD compared to the UD-GCMS procedure for all analytes with an exception of MDPV, which showed comparable LOD results. Due to the wide availability of GC-MS in hospital laboratories our method is a notable evolutionary improvement to identify designer stimulant overdoses or exposures.

Keywords: Designer Drugs, 2C Amines, GC-MS

P63

Hydrolysis Optimization for Eight Cannabinoids in Human Urine Employing Recombinant β-Glucuronidase

Karl B. Scheidweiler*, Cristina Sempio, Allan J. Barnes, Marilyn A. Huestis; Chemistry and Drug Metabolism Section, Clinical Pharmacology and Therapeutics Research Branch, Intramural Research Program, NIDA, NIH, Baltimore, MD

Background/Introduction: Distinguishing recent cannabis intake during treatment, workplace, clinical or forensic urine testing is confounded by prolonged excretion in chronic cannabis users. Previous studies for Δ9-tetrahydrocannabinol (THC), 11-hydroxy-THC (11-OH-THC) and 11-nor-9-carboxy-THC (THCCOOH) in urine after tandem alkaline/glucuronidase hydrolysis were unable to differentiate recent cannabis intake in a single specimen.

Objective: To develop an optimized hydrolysis approach for THC, 11-OH-THC, THCCOOH, cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), tetrahydrocannabivarin (THCV) and 11-nor-9-carboxy-THCV (THCVCOOH) in urine employing recombinant β-glucuronidase (EBG; 50,000 U/mL, Kura Biotec, Puerto-Vara, Chile) to evaluate these analytes as indicators of recent cannabis intake.

Method: Urine samples were collected from a healthy cannabis user who provided written informed consent to participate in a National Institute on Drug Abuse (NIDA) Institutional Review Board-approved study. The study characterized cannabinoid pharmacokinetics and novel markers of cannabis intake following smoking within 10 min of a single cannabis cigarette containing THC 6.9% (w/w). All urine specimens were stored at -20°C prior to analysis. Urine specimens collected 0.4 and 2.5 h after smoking were combined to create a single pool for optimizing hydrolysis. Time (2, 16 and 40 h), temperature (25, 37 and 46ºC), enzyme amount (500, 1000 and 2000 U/sample), buffer molarity (0.5, 1 and 2M) and pH (5.8, 6.8 and 7.8) were evaluated in quadruplicate along with samples analyzed without hydrolysis. 200 µL urine was fortified with 20 µL internal standard, 50 µL sodium phosphate buffer and 40 µL recombinant β-glucuronidase before incubation at 25, 37 or 46ºC for 2, 16 or 40 hr. 620 µL acetonitrile was added before centrifugation; 550 µL was collected from the upper layer and transferred to a clean deep well plate containing 200 µL 5% formic acid in water. The plate was placed on a Tecan Freedom EVO 100 and the solution was pumped four times through a DPX WAX-S tip (1 mL with 20 mg resin and 40 mg salt). 60 µL upper layer was collected and transferred to a clean plate with 0.5 mL glass inserts containing 140 µL 0.15% formic acid in water. 45 µL was injected onto a Shimadzu UFLCxr liquid chromatograph- SCIEX 5500 QTRAP mass spectrometer system. Gradient elution at 0.5 mL/min was performed on a UCT Selectra DA column (100 mm x 2.1 mm; 3 µm) with 0.15% formic acid in A) water and B) acetonitrile. Positive mode ESI mass spectrometric data were acquired in MRM mode. Analyte peak areas were evaluated with ANOVA and Bonferroni post-hoc comparisons ($p<0.05$).

Result: All analyte peak areas increased after hydrolysis ($F_{15,48}$=9.7-79.9). There were no significant differences between hydrolysis conditions for THC, CBD, CBN, THCV and THCVCOOH. THCCOOH and 11-OH-THC peak areas were significantly increased after 16 vs. 2 h, while CBG peak areas were significantly decreased after 16 vs. 2 hr. 11-OH-THC peak areas were significantly decreased at 25 and 46ºC compared to 37ºC; significantly decreased 11-OH-THC peak areas were observed at pH 7.8 compared to pH 5.8 and 6.8. 11-OH-THC peak areas significantly increased as amount of enzyme increased. 2M sodium phosphate buffer yielded less peak area variability than 0.5 and 1M buffer for all analytes.

Conclusion/Discussion: Optimized cannabinoids hydrolysis in urine with recombinant β-glucuronidase was achieved with: 2000 Units of enzyme/sample, 2M sodium phosphate buffer, pH 6.8 incubated at 37ºC for 16 hr. Analyzing specimens collected during our controlled cannabis administration pharmacokinetics study via a fully validated liquid chromatography tandem mass spectrometry method incorporating these hydrolysis conditions enables evaluating whether urinary CBD, CBN, CBG, THCV or THCVCOOH can be employed as indicators of recent cannabis intake.
This work was funded by NIDA-IRP, NIH.

Keywords: Cannabis, Urine, Enzyme Hydrolysis

P64

Method Validation for Quantification of Sixteen Benzodiazepines in Blood, and Confirmation of the Same in Urine, Using LC-MS/MS

Protiti Sarker*, Rachel Callaway, Robert Armenta, Rong-Jen Hwang; Toxicology Bureau – Scientific Laboratory Division – New Mexico Department of Health, Albuquerque, NM

Background/Introduction: Benzodiazepines, a class of CNS depressant drugs used to treat anxiety and sleep disorders, are among the most commonly prescribed drugs in the world. They are often encountered in DUID casework and postmortem samples and their use in drug facilitated sexual assaults (DFSA) is well documented. Many benzodiazepines and their active metabolites are difficult to detect using conventional gas chromatography/mass spectrometry (GC/MS) methods. This is primarily due to the poor suitability for the GC/MS system and their low therapeutic dosages. A method was developed to harness the sensitivity of a liquid chromatography tandem mass spectrometry (LC/MS-MS) system. This enabled quantitation of sixteen benzodiazepines in blood and confirmation in urine using fourteen deuterated internal standards to increase selectivity.

Objective: A solid phase extraction followed by liquid chromatography tandem mass spectrometry method was developed and validated for the simultaneous quantitation of alprazolam, alpha-hydroxyalprazolam, chlordiazepoxide, clonazepam, 7-aminoclonazepam, diazepam, flunitrazepam, 7-aminoflunitrazepam, flurazepam, desalkylflurazepam, lorazepam, midazolam, nordiazepam, oxazepam, temazepam, and triazolam in blood and urine. The SWGTOX guideline were followed.

Method: Deuterated internal standards were added to samples followed by 5 mL of pH 4.5 sodium acetate buffer. Samples were sonicated, centrifuged and applied to SPEWare Cerex Clin II SPE columns preconditioned with ethyl acetate and methanol. The columns were washed with potassium carbonate buffer pH 9 and deionized water before elution with ethyl acetate: ammonium hydroxide (98:2). Samples were reconstituted in LCMS grade methanol before injection onto an Agilent 1200 series LC coupled to an Agilent 6410 Triple Quadrupole Mass Spectrometer. HPLC separation was attained by using water with 0.1% formic acid and a gradient of methanol over an Agilent Zorbax C18 HPLC column (1.8 µM X 2.1 x 50 mm) at 35º C and analyzed using dynamic multiple reaction monitoring. Validation was performed using SWGTOX guidelines for calibration model fits, accuracy and precision, sensitivity measured by the limit of detection (LOD) and limit of quantitation (LOQ), carryover, interference, ion suppression/enhancement, dilution integrity and stability.

Result: All analytes were determined to fit to a linear 1/x weighted curve with an $R^2 \geq 0.9975$ with an LOQ of 25 ng/mL and an upper limit of quantitation of 1000 ng/mL. The LOD varied from < 1 ng/mL (alprazolam, 7-aminoclonazepam, flurazepam, midazolam and oxazepam) up to 9 ng/mL (chlordiazepoxide, flunitrazepam). Percent accuracy at three concentrations ranged from 93-108%, between run bias %CV of 2-8% and the largest within run precision was used to determine acceptability, which varied from 2-17%, all within acceptable CV values of ± 20 %. No significant carryover, interference from matrix effects or drugs of abuse, or ion suppression/enhancement effects were noted. Furthermore, samples proved stable over 5 days and were successfully quantitated at dilutions of 1:2, 1:4 and 1:10.

Conclusion/Discussion: A method for the quantitation of sixteen benzodiazepines in postmortem and ante- mortem blood and confirmation in urine was successfully developed and validated using SPE and LC/MS-MS. The sensitivity of this method has allowed us to successfully confirm and quantitate many benzodiazepines and metabolites that were routinely not seen by GC/MS in our laboratory. Additionally, this method was developed using a methanol mobile phase instead of acetonitrile which has a large cost advantage.

Keywords: Benzodiazepines, LC-MS/MS, Validation

P65
LC-MS/MS Method Development Challenges for the Analysis of 43 Anxiety Medications and Metabolites

Ty Kahler*, Sharon Lupo, Frances Carroll; Restek Corporation, Bellefonte, PA

Background/Introduction: The use of liquid chromatography coupled with mass spectrometry (LC-MS/MS) has become a routine method of analysis in forensic and clinical labs. LC-MS/MS provides sensitivity, speed, and specificity when analyzing drugs in complex biological matrices, such as blood and urine. Anxiety medications, such as benzodiazepines, muscle relaxers, hypnotics, sedatives, z-drugs, and barbiturates are used to treat a variety of conditions and are often abused in conjunction with other drugs. The presence of isomers and the need to collect data in positive and negative ion modes can present the analyst with significant chromatographic challenges.

Objective: The intent of this study was to develop a comprehensive assay for medications used to treat anxiety including benzodiazepines, muscle relaxers, hypnotics, sedatives, z-drugs, and barbiturates on the Raptor™ Biphenyl column.

Method: During development, the anxiety medications were divided into 6 mixtures containing a total of 43 drugs and metabolites. These mixtures were diluted in water and injected into a Shimadzu Nexera UHPLC equipped with an AB SCIEX API 4500™ MS/MS. Detection was performed using electrospray ionization in positive and negative ion modes with multiple reaction monitoring (MRM). Multiple mobile phase combinations and additives were investigated and comparisons were made using scouting gradients to evaluate retention, resolution, and sensitivity.
The final optimized methods utilized water and methanol mobile phases modified with 0.1% formic under gradient conditions on Restek Raptor™ Biphenyl and Restek Raptor™ C18 2.7µm, 100 x 2.1mm columns. Both columns were equipped with EXP® 2.7µm, 5 x 2.1mm guard columns of the same phase.

Result: Through mobile phase investigations, it was found that although acetonitrile provided a faster run, the use of methanol improved peak capacity over the gradient. In addition, the inclusion of ammonium formate in the mobile phase reduced sensitivity over formic acid alone. Acidic mobile phases typically improved peak response for most analytes, including the barbiturates.
Barbiturates are weak acids in solution therefore negative ion mode is required for ionization. In addition, the barbiturates amobarbital and pentobarbital are positional isomers and must be chromatographically separated for accurate identification. Since the other drugs in the panel show better responses in positive ion mode, this assay can either be performed in a single run which employs polarity switching or with two separate analyses. Since the isomers can be extremely difficult to resolve, the separation was attempted on two different phases: the Raptor™ Biphenyl and the Raptor™ C18. Using the Biphenyl column, a combined analysis of anti-anxiety drugs and barbiturates was achieved with polarity switching. However, this method resulted in ~40% resolution between amobarbital and pentobarbital and also requires an LC-MS/MS with sufficient data speed to accomplish the analysis. It was found that near baseline resolution (~95%) of the barbiturate isomers could be accomplished by analyzing the barbiturates separately on the Raptor™ C18 column. The remaining anti-anxiety drugs could be quickly analyzed on the Raptor™ Biphenyl column.

Conclusion/Discussion: If only partial resolution between the barbiturate isomers is required, the Raptor™ Biphenyl column allows the combined analysis of anti-anxiety drugs and barbiturates in just 8 minutes. If resolution of barbiturate isomers is critical, anti-anxiety drugs and barbiturates can be analyzed separately. By using the Raptor™ C18 column for the barbiturates analysis, amobarbital and pentobarbital are almost completely resolved in 6 minutes, while the analysis of the anti-anxiety drugs can be completed on the Raptor™ Biphenyl column in 5.5 minutes. This simpler approach improves the resolution of barbiturate isomers and is suitable for slower mass spectrometers that lack the speed required for combined analysis.

Keywords: LC-MS/MS, Anxiety Medications, Barbiturates

P66

The Analysis of Emerging Drugs of Abuse: Updating an Existing Method with New Compounds

Ty Kahler, **Frances Carroll***, Sharon Lupo, Shun-Hsin Liang, Susan Steinike, Carrie Sprout, Paul Connolly; Restek Corporation, Bellefonte, PA

Background/Introduction: The determination of psychoactive drugs and their metabolites has become routine in many forensic toxicology laboratories. The optimization of analysis time, resolution between metabolites, method robustness, and the ability to add emerging compounds is of ultimate importance when developing an efficient method for validation. The Raptor™ Biphenyl column combines the speed of superficially porous particles (SPP) with the resolution of highly selective USLC® technology to give the analyst the ability to produce fast dilute and shoot methods while staying current with the ever changing landscape of illegal drugs.

Objective: Provide an expanded method for the fast and easy analysis of 22 synthetic cannabinoids, 12 metabolites, and salvinorin A resulting in complete resolution of isobars and separation from matrix interferences in diluted human urine.

Method: The method investigations were performed on a Waters Acquity I-class equipped with a Xevo TQ-S and a Shimadzu Nexera equipped with a SCIEX 4500. Both systems utilized electrospray ionization in positive ion mode. Standards were prepared in human urine and were diluted 3x in a 0.2 µm PVDF Thomson SINGLE StEP® Filter Vial with 50:50 water:methanol prior to analysis. Data was collected with MRM windows of approximately ± 30 seconds. Chromatographic optimization resulted in complete resolution of isobars and separation from major matrix interferences of a representative pooled urine sample. Water and acetonitrile mobile phases modified with 0.1% formic acid were used under gradient conditions on a Restek Raptor™ Biphenyl 2.7µm, 50 x 3.0mm column equipped with a Raptor™ Biphenyl EXP 2.7µm, 5 x 3.0mm guard.

Result: Chromatographic separation is essential for analyzing synthetic cannabinoids JWH-018 and JWH-073 and their metabolites due to the presence of multiple positional isomers among the mono-hydroxylated metabolites. Since these positional isomers have identical molecular weights and very similar fragmentation patterns, they are indistinguishable by MS/MS detectors and chromatographic resolution is required for positive identification.
Previously a method was presented for the comprehensive screen of 17 synthetic cannabinoids, 12 metabolites and 5 internal standards with a cycle time of 5 minutes. All positional isomers were resolved on the Raptor™ Biphenyl column making it possible for the most abundant metabolites from a given parent compound to be identified in authentic samples.

Today, laboratories are faced with the difficult task of keeping up with the ever-growing list of synthetic cannabinoids illicit drug makers produce to avoid legal classification and detection. In an effort to determine the ability of the original method to keep pace with the rapidly changing list, 5 emerging synthetic cannabinoids (i.e. AB-PINACA, AB-FUBINACA, PINACA, 5F-PB-22, and PB-22) and salvinorin A were prepared in human urine and analyzed using the same methodology.

Conclusion/Discussion: The analysis of synthetic cannabinoids and their metabolites can be a difficult and challenging task. The Raptor™ Biphenyl provides solutions to the chromatographic and validation issues surrounding this analysis. It has the ability to provide highly retentive, selective, and rugged reversed-phase separations, allowing for the simultaneous analysis of 22 synthetic cannabinoids, 12 metabolites, and salvinorin A. It has been demonstrated that analyte lists can easily be expanded as new synthetic cannabinoids are introduced. The speed of SPP allows analysis times to become shorter. The unique selectivity of the biphenyl phase allows isomer separation to be easily achieved.

Keywords: Synthetic Cannabinoids, Salvinorin A, LC-MS/MS

P67
A Multi-Class Drug and Metabolite Screen of 231 Analytes by LC-MS/MS

Shun-Hsin Liang, Frances Carroll, Sharon Lupo, Carrie Sprout, Ty Kahler, Paul Connolly, **Alicia Sullivan*;** Restek Corporation, Bellefonte, PA

Background/Introduction: The use of pain management drugs has been steadily increasing. As a result, labs are seeing an increase in patient samples that must be screened for a wide variety of drugs to prevent drug abuse and to ensure patient safety and adherence to their medication regimen. Therapeutic drug monitoring can be challenging due to the low cut-off levels, potential matrix interferences and isobaric drug compounds. To address these challenges, many drug testing facilities are turning to liquid chromatography coupled with mass spectrometry (LC-MS/MS) for its increased speed, sensitivity, and specificity. In this example, a method was developed for a multi-class drug and metabolite screen containing 231 compounds.

Objective: The intent of this study was to develop a screening method for multi-class drug panel including opioids, anti-anxiety, anti-depressants, anti-epileptics, anti-psychotics, barbiturates, cannabinoids, NSAIDs, stimulants, and hallucinogens using the Raptor™ Biphenyl column.

Method: There are many challenges one must consider when developing a large screening assay. Experiments explored mobile phase and sample diluent effects, isomer resolution, and drug interferences. Analytes were diluted in water and injected into a Shimadzu Nexera UHPLC equipped with an AB SCIEX API 4500™ MS/MS. Detection was performed using electrospray ionization in positive and negative ion modes using scheduled multiple reaction monitoring (MRM).

Result: During mobile phase investigations, it was found that methanol provided the best retention of early eluting opioids, such as morphine, oxymorphone, and hydromorphone. The addition of ammonium formate to the mobile phase was required to retain the stimulants nicotine and norcotinine, while acidic mobile phases typically improved peak response for most analytes.
Isobar separation on the Raptor™ Biphenyl is largely influenced by the use of protic or aprotic organic solvents (e.g., methanol or acetonitrile, respectively) due to the column's polarizability. The opioids dihydrocodeine and noroxycodone can be easily separated when an acetonitrile mobile phase is used. However, the resolution is lost when the organic solvent is switch to methanol. In addition to isobaric interferences, structurally similar compounds from different drug classes can form identical products resulting in analogous transitions, as is the case for carbamazepine (ant-psychotic) and eslicarbazepine (anti-epileptic).
Due to the large number of compounds screened, it is imperative that stock standards are prepared at sufficient concentrations to allow for dilution into weaker solvents. If the organic solvent content is too high, early-eluting peaks appear broad and distorted and could negatively impact peak identification.

Conclusion/Discussion: The Raptor™ Biphenyl column was demonstrated to be excellent for the screening of 231 multi-class drugs (including metabolites) with balanced retention and a final optimized gradient of 10 minutes, and a total cycle time of 12 minutes. The optimized method utilized water and methanol mobile phases modified with 0.1% formic acid and 2 mM Ammonium formate under gradient conditions on a Restek Raptor™ Biphenyl 2.7µm, 100 x 2.1mm column equipped with a Raptor™ Biphenyl EXP® 2.7µm, 5 x 2.1mm guard column. Of the mixture of analytes, 209 were analyzed in positive ion mode, and 22 were analyzed in negative ion mode.

Keywords: LC-MS/MS, Raptor™ Biphenyl, Therapeutic Drug Monitoring

P68
Analysis of Fentanyl and Its Analogues in Human Urine by LC-MS/MS

Carrie Sprout*, Shun-Hsin Liang, Frances Carroll, Sharon Lupo, Ty Kahler, Paul Connolly; Restek Corporation, Bellefonte, PA

Background/Introduction: Synthetic opioid drugs, such as fentanyl and sufentanil, have very high analgesic potency. Abuse of these prescription opioids and their illicit analogue, acetyl fentanyl, is a growing public health problem. In this study, a simple dilute and shoot method was developed with an analysis time of less than 3.5 minutes for fentanyl, norfentanyl, acetyl fentanyl, and sufentanil in human urine by LC-MS/MS using the Raptor™ Biphenyl column.

Objective: Provide a simple, fast, and sensitive measurement of fentanyl, its metabolite, and analogues in human urine.

Method: Pooled human urine was fortified with the analytes. The urine sample was diluted 5-fold in a water/methanol solution with the addition of internal standards (fentanyl-d5, norfentanyl-d5, sufentanil-d5, acetyl fentanyl-$^{13}C_6$) prior to injection on the Raptor™ Biphenyl column (50x2.1mm, 5µm). The mobile phases used were 0.1% formic acid in water (aqueous phase) and 0.1% formic acid in methanol (organic phase) and the chromatographic separation was achieved with a gradient elution of 30% - 80% organic phase in 2 minutes. The analysis was performed on a Waters ACQUITY UPLC® I-Class System coupled with a Waters Xevo TQ-S mass spectrometer using electrospray ionization in positive ion mode.

Result: All four analytes were completely resolved on the Raptor™ Biphenyl column with a 2-minute gradient elution. No matrix interference was observed for quantitation. The calibration linearity ranged from 0.05 to 50 ng/mL for fentanyl, acetyl fentanyl, and sufentanil; and 0.25 to 50 ng/mL for norfentanyl with % deviation of less than 10.0% and the R^2 of $\geq$ 0.999. The LLOQ was 0.25 ng/mL for norfentanyl, and 0.05 ng/mL for fentanyl, acetyl fentanyl, and sufentanil in urine. Three levels of QC samples (0.75, 4.0, and 20.0 ng/mL) were analyzed for accuracy and precision. Based on three independent experiments conducted on multiple days, the mean accuracy values ranged from 94 to 110% of the nominal concentrations for all compounds and the %RSD ranged from 0.2 to 9.2%.

Conclusion/Discussion: An easy dilute and shoot method was developed for the quantitative measurement of fentanyl, its metabolite, and analogues in human urine. The analytical method was demonstrated to be fast and sensitive with great accuracy and precision for urine sample analysis. It also shows that the Raptor™ Biphenyl column is well suited for the analysis of synthetic opioid compounds for forensic toxicology.

Keywords: Fentanyl Analogs, LC-MS/MS, Urine

P69

LC/MS/MS Analysis of Urinary Benzodiazepines and Z-drugs Via a Simplified, Mixed-mode Sample Preparation Strategy

Jonathan P. Danaceau*, Erin E. Chambers; Waters Corporation, Milford, MA

Background/Introduction: Benzodiazepines are commonly prescribed drugs used for their sedative, anxiolytic, and hypnotic properties. So-called "Z-drugs" (zolpidem and zopiclone) are commonly used sleep aids that act in a similar manner to benzodiazepines. While the use of LC/MS/MS for benzodiazepine analysis has increased in recent years, many published techniques still rely on labor intensive liquid-liquid extraction techniques. This method analyzes 18 benzodiazepine drugs and metabolites, along with zolpidem, zopiclone and N-desmethyl zopiclone.

Objective: The objective of this study was to develop a simplified sample preparation and LC/MS/MS analysis strategy for these compounds. Strong cation exchange micro elution plates were used to rapidly extract these compounds from urine samples. All sample preparation steps, including enzymatic hydrolysis, were performed within the wells of the µElution plates, and the extraction method is simplified by eliminating conditioning and equilibration steps.

Method: 200 µL of urine was added to individual wells of a mixed-mode strong cation exchange µElution SPE plate, along with internal standards, hydrolysis buffer and *β*-glucuronidase enzyme. 20 deuterated internal standards were used for quantification. Samples were incubated for 1 hr. at 50 °C. After incubation, samples were quenched with 200 µL of 4% H_3PO_4 and directly loaded onto the sorbent bed by vacuum. All samples were subsequently washed with 200 µL of 0.02 N HCl, and 200 µL of 20% MeOH. Samples were eluted with 2 x 25 µL of 60:40 ACN:MeOH containing 5% strong ammonia solution and then diluted with 100 µL of sample diluent (2% ACN:1% formic acid in water). 5 µL of each sample was injected and analyzed by UPLC/MS/MS using a Waters' Cortecs C18+ column (1.6 µm; 2.1 x 100) and a Xevo® TQ-S micro mass spectrometer. Mobile phases A and B consisted of water and acetonitrile, respectively, each containing 0.1% formic acid. The solvent ramp started at 10% MPB and increased to 50% over 5 minutes, followed by a wash at 95% B and reequilibration. The total cycle time was 7 minutes.

Result: All compounds eluted in less than 5.2 minutes with baseline separation achieved between all relevant analytes. Extraction recoveries ranged from 76-102% with an average of 91%, demonstrating excellent extraction efficiency. The recoveries were consistent, with coefficients of variation (%CVs) ranging from 5.2% to 15%, with a mean of 8.6%. Matrix effects ranged from -45% to 30% with an average amplitude of 18%.
Calibration curves ranged from 0.5 ng/mL through 500 ng/mL for all compounds. All compounds had R^2 values of 0.997 or greater. Within-batch QC results showed excellent accuracy and precision. With one exception, all compounds were accurate to within 15% of target values and had %CVs of less than 15% (N=6). The between-batch results had accuracies within 10% of target values and %CVs less than 10% at all 4 QC levels (N=4 batches). SWGTOX guidelines were used to determine accuracy (bias) and precision. Limits of quantification were 0.5 ng/mL for all compounds and were defined as the lowest point at which bias and %CV were <15%, S/N > 5 and peak intensities were 10x those seen in blank matrix samples.

Conclusion/Discussion: This rapid and simplified SPE protocol and LC/MS/MS method for the analysis of urinary benzodiazepines and metabolites enables the entire pretreatment protocol to be conducted within the well of the SPE plate. This eliminates time consuming and error prone transfer steps. The elimination of conditioning and equilibration had no effect on extraction efficiency. Furthermore, the ability to elute in only 50 µL eliminates the need for evaporation and reconstitution. This results in a highly efficient and reproducible method that provides excellent efficiency, accuracy and precision for the analysis of this highly prescribed class of compounds.

Keywords: Sample Preparation, Benzodiazepines, LC/MS/MS

P70

Degradation of Opiates During Acid Hydrolysis Leads to Reduced Recovery Compared to Enzymatic Hydrolysis

Pongkwan Sitasuwan[1], **Cathleen Melendez*[1]**, Margarita Marinova[1], Kaylee R. Mastrianni[2], Alicia Darragh[3], Emily Ryan[3], L. Andrew Lee[1]; [1]Integrated Micro-Chromatography Systems, LLC, Columbia, SC, [2]Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC, [3]LabSource, LLC, Greenville, SC

Background/Introduction: Opiates are routinely monitored in clinical laboratories to ensure patients are compliant with their pain management prescriptions or to test for illicit drug use. To facilitate drug monitoring, laboratories often pre-treat patient samples with concentrated acid or enzyme to liberate glucuronides from parent compounds to simplify the detection of drugs via mass spectrometry. Past reports indicate acid-catalyzed hydrolysis as a more effective means of liberating glucuronides from opiates than enzyme catalyzed hydrolysis. Here we present that acid hydrolysis effects a side reaction that converts oxycodone to oxymorphone and hydrocodone to hydromorphone, resulting in possible false negatives for oxycodone and hydrocodone. Uncontrolled hydrolysis events are typical for acid-catalyzed reactions, especially in the presence of high heat, and acid-catalyzed hydrolysis events are well known for degrading the heroin metabolite (6-monoacetylmorphine). These side reactions should be cautiously noted for clinical testing laboratories hydrolyzing samples with concentrated acid at high temperatures.

Objective: Demonstrate that acid catalyzed demethylation of oxycodone and hydrocodone occurs. Compare two different hydrolysis methods and monitor the changes in opiate concentrations. Monitor hydrolysis efficiencies by testing drug free urine spiked with oxymorphone-, hydromorphone-, and codeine-glucuronides. Further validate the hydrolysis efficiencies using patient urine samples that tested positive for oxycodone and hydrocodone.

Method: Acid catalyzed byproducts of oxycodone and hydrocodone were monitored by replacing human urine with water. A published protocol was followed in which hydrochloric acid was added to opiate fortified water and heated at 95°C up to 90 minutes. After sample neutralization and centrifugation, the acid-treated samples were analyzed using LC-qTOF and LC-MS/MS. Acid hydrolysis was performed on eighteen authentic patient urine samples that tested positive for oxycodone and hydrocodone. The same hydrolysis procedure was used with the exception that all patient samples treated with acid were heated for 90 minutes. After neutralization and centrifugation, the samples were extracted and analyzed using LC-MS/MS. For comparative analysis, the positive patient samples were hydrolyzed with a genetically modified β-glucuronidase, IMCSzyme®, at 55°C for 30 minutes. Similar to acid hydrolyzed samples, the enzyme hydrolyzed samples were extracted and then analyzed using LC-MS/MS.

Result: Acid hydrolysis of fortified water showed an increase from 0 to 0.13 in the area ratios of both oxymorphone and hydromorphone in relation to internal standard and 27% and 16% decreases in oxycodone and hydrocodone over 90 minutes, respectively. The analysis of eighteen patient samples showed that recoveries of oxycodone, oxymorphone, hydrocodone, and hydromorphone were consistently lower for acid hydrolysis, reflecting decreases of 82%, 67%, 90%, and 54%, respectively when compared to enzyme hydrolysis results. In addition, acid treatment yielded eight false negatives and one false positive for either oxycodone or hydrocodone.

Conclusion/Discussion: Acid hydrolysis of oxycodone and hydrocodone results in a demethylation event which converts these opioids to oxymorphone and hydromorphone, respectively. More accurate quantitative analysis may be achieved by performing a different hydrolysis method, such as one utilizing the IMCSzyme® β-glucuronidase, to monitor for opiate usage.

Keywords: Acid Hydrolysis, IMCSzyme®, β-Glucuronidase

P71

Chiral Analysis of Methamphetamine in Oral Fluid Samples: A Method to Distinguish Licit from Illicit Drug Use

Damon Borg*, Elizabeth Kolb, Alisa Valentino, Richard Stripp; Cordant Health Solutions, Denver, CO

Background/Introduction: Methamphetamine (MAMP) is a popular illicit drug abused for its central nervous system (CNS) stimulating effects. MAMP is also used therapeutically in the treatment of overeating disorders, narcolepsy, attention deficit disorder, in OTC products to ease nasal congestion, and can be detected as a metabolite of medications such as selegiline and benzphetamine. MAMP is excreted from the body in two enantiomeric forms, dextrorotary (*d*-MAMP) or levorotary (*l*-MAMP). The compounds are similar in chemical structure, simply differing in the orientation of functional groups around the asymmetric carbon. The other major difference between enantiomers concerns their pharmacologic effects. *d*-MAMP elicits CNS stimulant effects while *l*-MAMP produces vasoconstriction with little CNS activity. In part because of the availability of *l*-MAMP in OTC nasal inhalers, forensic guidelines require a sample to contain greater than 20% *d*-MAMP to consider illicit drug use when interpreting results. Standard analytical methods readily detect MAMP in biological specimens but cannot resolve the enantiomeric composition of the sample. Specialized analytical techniques based on chiral separation of the enantiomers are required to differentiate *d*-MAMP from *l*-MAMP. A large body of literature has characterized chiral *d/l*-MAMP analysis in urine specimens using various procedures and highlighting representative data from authentic urine drug tests. However, methods for chiral *d/l*-MAMP analysis of oral fluid samples and representative data from MAMP positive donor samples are limited. As oral fluid drug testing becomes more popular for routine workplace drug testing, this information is particularly relevant.

Objective: Our laboratory sought to develop and validate a method for the analysis of oral fluid specimens for *d/l*-MAMP using a chiral derivatizing agent and traditional reverse phase liquid chromatography tandem mass spectrometry (LC/MSMS).

Method: MAMP was extracted from dilute oral fluid samples collected with Quantisal devices (Immunalysis Corp, Pomona CA) using Strata-XC solid phase extraction cartridges (Phenomenex, Torrance, CA) and derivatized with Marfey's reagent (Sigma-Aldrich, St. Louis MO). Chromatographic separation was achieved using an Agilent 1290 liquid chromatograph equipped with Zorbax Eclipse Plus C18 columns (2.1mmx50mm 1.8um). Mobile phases consisted of 0.1% formic acid (A) and 100% methanol (B). The chromatographic run time was 3.4 minutes. Positive identification was made using an Agilent 6460 triple quadrupole mass spectrometer with a Jetstream source operating in positive ion mode with the following common parameters: drying gas temperature 350°C, sheath gas temperature 400°C, drying gas flow 10 L/min, sheath gas flow 11 L/min, nebulizer pressure 50 psi, capillary voltage 4000 V, and nozzle voltage 1000 V. All qualifier ion ratios were determined to be within +/- 20% of calibrator qualifier ion ratios.

Result: Linearity, accuracy and precision, recovery, matrix effects, and specificity of the method were all within acceptable criteria. Interday (N=45) accuracy of quality control samples ranged from 89-108%, while precision (RSD, N=45) ranged from 5-8%. The recovery of target analytes from spiked oral fluid samples ranged from 89% to 106%. Finally, having previously tested positive for methamphetamine using non-chiral analysis, 54 de-identified authentic oral fluid samples were analyzed using this validated method. 94% of all samples tested positive for *d*-MAMP at greater than 20%.

Conclusion/Discussion: Illicit MAMP can be distinguished from licit MAMP through chiral analysis of samples testing positive for MAMP using standard analytical methods. This method was developed and validated to analyze oral fluid samples for *d/l*-MAMP using chiral derivatizing agents and traditional LC/MSMS. Application of the method to authentic oral fluid samples indicated 94% contained *d*-MAMP at greater than 20%. This method will provide laboratories with a means to accurately assess the enantiomeric composition of MAMP positive oral fluid samples.

Keywords: Methamphetamine, Chiral Analysis, Oral Fluid

P72
Prescription Opioids. V. Profiling Oxymorphone in Urine

Charles P. LoDico*[1], Ronald R. Flegel[1], Anne Z. DePriest[2,3], Rebecca Heltsley[2], David L. Black[2,4], John M. Mitchell[5], Edward J. Cone[6]; [1]Substance Abuse and Mental Health Services Administration (SAMHSA), Division of Workplace Programs (DWP), Rockville, MD, [2]Aegis Sciences Corporation, Nashville, TN, [3]University of Tennessee Health Science Center, College of Pharmacy, Memphis, TN, [4]Vanderbilt University, Department of Pathology, Microbiology, and Immunology, Nashville, TN, [5]RTI International, Research Triangle Park, NC, [6]Johns Hopkins School of Medicine, Baltimore, MD

Background/Introduction: Oxymorphone (OM) is prescribed in the United States for the treatment of pain, and is also a metabolite of oxycodone. It undergoes metabolism primarily to conjugated OM, with minor pathways producing noroxymorphone (NOM) and other metabolites. As of May 2015, OM has been included in the proposed Mandatory Guidelines for Federal Workplace Drug Testing Programs.

Objective: Measure OM concentrations in urine following oral administration to drug-free human subjects under controlled clinical conditions.

Method: Twelve healthy, drug-free subjects (8 males/4 females) were orally administered a single dose of one 10 mg OM controlled release tablet. The study was approved by an Institutional Review Board and subjects provided informed consent. Pooled urine specimens were collected at timed intervals for 54 hours post-dose. Specimens were frozen and shipped to the laboratory for analysis by liquid chromatography tandem mass spectrometry (LC-MS-MS) using a 50 ng/mL limit of quantitation (LOQ) for OM and NOM. Analysis was conducted in hydrolyzed and non-hydrolyzed urine.

Result: OM and NOM were detected in 89.9% and 13.5% of hydrolyzed urine specimens, respectively. Free OM was detected at much lower concentrations in 8.1% of non-hydrolyzed specimens, for six subjects; NOM was not detected in any non-hydrolyzed specimen. In hydrolyzed urine, total OM was initially detected at a mean (range) of 2.4 h (2 – 4 h), with NOM initially detected at 8.3 h (6 – 10 h). Free OM was initially detected at 9.3 h (6 – 14 h). Mean maximum concentrations were 3,560 ng/mL (271 – 9,892 ng/mL) for total OM, 89 ng/mL (59 – 178 ng/mL) for total NOM, and 84 ng/mL (50 – 192 ng/mL) for free OM. Maximum concentrations were observed at a mean of 9 h (4 – 24 h) for total OM, 12 h (8 – 28 h) for total NOM, and 10 h (6 – 14 h) for free OM. NOM presence was always accompanied by OM in hydrolyzed urine, with a mean OM:NOM ratio of 41.6. At a 100 ng/mL threshold, mean detection times were 47.5 h (28 – 54 h) for total OM, 3.0 h (0 – 28 h) for total NOM, and 0.7 h (0 – 8 h) for free OM. At a 50 ng/mL threshold, mean detection times were 50.7 h (32 – 54 h) for total OM, 11.0 h (0 – 28 h) for total NOM, and 5.8 h (0 – 14 h) for free OM.

Conclusion/Discussion: The majority of a single controlled-release dose of OM is excreted in urine as conjugated metabolites. NOM is detected at much lower concentrations, and very little drug is excreted as free OM. Testing for OM should therefore include hydrolysis or detection of OM glucuronide. The detection period of a single dose of OM may be prolonged for approximately two days when using 50-100 ng/mL thresholds.

Keywords: Urine, Oxymorphone, Detection Times

P73
Prescription Opioids VI - Profiling Hydromorphone in Urine

Ronald R. Flegel*[1], Charles P. LoDico[1], Anne Z. DePriest[2,3], Rebecca Heltsley[2], David L. Black[2,4], John M. Mitchell[5], Edward J. Cone[6]; [1]Substance Abuse and Mental Health Services Administration (SAMHSA), Division of Workplace Programs (DWP), Rockville, MD, [2]Aegis Sciences Corporation, Nashville, TN, [3]University of Tennessee Health Science Center, College of Pharmacy, Memphis, TN, [4]Vanderbilt University, Department of Pathology, Microbiology, and Immunology, Nashville, TN, [5]RTI International, Research Triangle Park, NC, [6]Johns Hopkins School of Medicine, Baltimore, MD

Background/Introduction: Hydromorphone (HM) is available in the United States as a prescription drug, and like other opioids, is subject to abuse. It is also a metabolite of hydrocodone, and a minor metabolite of morphine. In 2015, the Substance Abuse and Mental Health Services Administration (SAMHSA) included hydromorphone in the proposed Mandatory Guidelines for Federal Workplace Drug Testing Programs.

Objective: Measure HM concentrations in urine following oral administration to drug-free human subjects under controlled clinical conditions.

Method: Twelve healthy, drug-free subjects (8 males/4 females) were administered a single oral dose of one 8 mg HM controlled release tablet. The study was approved by an Institutional Review Board and subjects provided informed consent. Pooled urine specimens were collected at timed intervals for 54 hours post-dose. Specimens were frozen and shipped to the laboratory for analysis by liquid chromatography tandem mass spectrometry (LC-MS-MS) using a 50 ng/mL limit of quantitation (LOQ) for free and "total" HM in non-hydrolyzed and hydrolyzed urine, respectively.

Result: Total HM was detected in 85.3% of hydrolyzed urine specimens collected post-dose, whereas free HM was detected in 47.6% of non-hydrolyzed urine specimens. Total HM was initially detectable at a mean (range) of 6.3 h (4 – 10 h) following dosing. Initial detection of free HM occurred later, at a mean of 9.6 h (6 – 24 h). The mean maximum concentration of total HM in hydrolyzed urine was 1918 ng/mL (357 – 3937 ng/mL), occurring at 20 h (10 – 28 h). The mean maximum concentration of free HM in non-hydrolyzed urine was 237 ng/mL (69 – 593 ng/mL), occurring at 19 h (10 – 24 h). The mean detection time for total HM was 52.3 h (48 – 54 h) at both 50 ng/ml and 100 ng/mL thresholds; eleven subjects were still positive at their final collection interval. The mean detection time for free HM was 38.0 h (0 – 52 h) at a 50 ng/mL threshold, and 26.0 h (0 – 52 h) at a 100 ng/mL threshold.

Conclusion/Discussion: Testing requirements for HM should include hydrolysis or detection of the conjugated metabolite, as only minor amounts are excreted as free HM. At sensitive thresholds of 50 to 100 ng/mL, HM detection may be prolonged for several days following ingestion of a single controlled release dose.

Keywords: Urine, Hydromorphone, Detection Times

P74

A Comparison of Barbiturate Screening Results Using Enzyme Immunoassay or a Highly Automated SPE-MS/MS Analysis

Janet Putnam*, Gregory L. McIntire, Erin C. Strickland; Ameritox, LLC, Greensboro, NC

Background/Introduction: Screening, the qualitative analysis of the presence of an analyte or family of analytes, has largely been the province of enzyme immunoassay (EIA). It is easy to run, relatively inexpensive, and provides rapid results. However, EIA tests are often cross reactive with any number of analytes and hence false positives can be a problem that is only resolved via confirmation testing by mass spectrometry. Liquid Chromatography – tandem mass spectrometry (LC-MS/MS) is extremely selective but hampered by the length of time required for LC separation. Given the qualitative nature of "screening", the use of a highly automated solid phase extraction – MS/MS technology (SPE-MS/MS) (e.g., "Rapid Fire®") affords rapid analysis, comparable costs/test, and more selective data; e.g., fewer false positives.

Objective: This work compares the results of screening barbiturates by EIA with the same samples screened using SPE-MS/MS. Assessments of time of analysis, per cent false positives, and fidelity between tests were examined to vet the SPE-MS/MS as an acceptable alternative to EIA for screening barbituates.

Method: Over 1000 patient urine specimens were submitted for qualitative testing by EIA and by SPE-MS/MS. Phenobarbital, butalbital, pentobarbital, and secobarbital were purchased from Cerilliant, along with the internal standard (butalbital D5). A DRI® Barbiturate assay kit was purchased from Microgenics using secobarbital to calibrate the screening assay. Samples were screened on an Olympus 5400 or an Olympus 640 for enzyme immunoassay analysis. The RapidFire SPE-MS/MS method development studies were performed on an Agilent 6460 with an Agilent RapidFire 300 front end. Urine samples were diluted ten-fold in a buffered IS diluent and were processed with a C18 cartridge before introduction into the mass spectrometer for analysis. The SPE-MS/MS method was a daily production method of which one day's results were applied to and compared to EIA screening results. For this study, the reporting cutoff for both the SPE-MS/MS and the EIA kit was 50 ng/mL.

Conclusion/Discussion: Comparison of the EIA screen with the SPE-MS/MS screen demonstrated similar patient results. When used in conjunction with confirmatory analysis, it was observed that the EIA screen produced about 1.5% more false positives than the SPE-MS/MS screen. The consumables cost for EIA testing is approximately $0.10/test while the same costs for the automated SPE-MS/MS test were below $0.05/test. Neither method required inordinate amounts of time to get a result; although EIA has an initial 30 min incubation period. Once past that incubation time and with continuous loading, the rate of sample result from EIA was approximately equal to the SPE-MS/MS. The capital equipment cost for an SPE-MS/MS system is lower than an EIA automated analyzer, however, the cost of EIA systems is often rolled into the cost of the kits thus spreading the capital cost across all kits used/yr. Employees that are already comfortable and trained on LC-MS/MS systems can easily be cross-trained to run the SPE-MS/MS system. The elevated level of EIA positives is consistent with the cross reactivity of that test and the selectivity of the MS/MS screen.

Keywords: Barbiturates, EIA, Mass Spectrometry

P75
Paroxetine and Paroxetine Metabolites in Human Urine Using LC/QTOF Analysis

Brandi R. Bridgewater*, Erin C. Strickland, Gregory L. McIntire; Ameritox, LLC, Greensboro, NC

Background/Introduction: Paroxetine (Paxil®) is a selective serotonin reuptake inhibitor prescribed to treat major depressive disorder, social anxiety disorder, panic disorder, and other syndromes. While the metabolism and excretion patterns of paroxetine have been documented from radiolabeling studies, the same information derived from liquid chromatography/time of flight mass (LC/QTOF) spectrometry has not been reported. This work examines patient positive samples for the types of metabolites and relative amounts of metabolites of paroxetine in urine by LC/QTOF.

Objective: The objective of this work was to evaluate the types of metabolites and apparent levels of paroxetine metabolites in human urine using LC/QTOF analysis.

Method: Human urine samples from individuals prescribed paroxetine and determined to be positive for paroxetine using a conventional liquid chromatography/tandem mass spectrometry (LC/MSMS) method were examined using an LC/QTOF method. This method has been developed to include a relatively long chromatography separation followed by positive mode and negative mode QTOF analysis of the peaks against a custom library of possible compounds. Paroxetine standard was obtained from Cerilliant while metabolite II was custom synthesized at better than 95% (Molecular 13C). Randomly selected authentic urine samples previously tested for paroxetine, using a validated dilute-and-shoot method, were collected and stored at 4°C until analysis. LC/QTOF analysis was carried out both by dilute and shoot and post hydrolysis with recombinant beta-glucuronidase (IMCS®) or combined beta-glucuronidase/sulfatase followed by solid phase extraction using SPEware Cerex PSAX 3 mL/35 mg cartridges. Analysis was completed on an AB Sciex 4600 Triple TOF coupled to an Agilent 1290 Infinity LC System with a Phenomenex Kinetex C18, 100 x 3.0 mm 1.7 µm column. The column temperature was 40°C and the cycle time for the method was 6 minutes.

Result: Analysis of patient samples demonstrated the presence of the same metabolites reported in earlier works. However, the relative intensities of these peaks differ from reported radiolabeled prevalence studies. The LC/QTOF data indicate that of the metabolites in the urine, approximately 60% arise from conjugated versions of the ring opened metabolites referred to as MI and MII consistent with the earlier work. Surprisingly, the parent drug (paroxetine) appears to be present at approximate 35% of the total metabolites present, inconsistent with earlier reports of <2%. These data suggest that paroxetine itself is either more efficiently ionized than the other metabolites under these conditions or the original radiolabeled studies are incorrect with respect to the relative distribution of metabolites. While custom synthesis yielded metabolite standards for this work, these standards are not yet currently commercially available. Hence routine analysis of MI/MII is difficult if not impossible at this time.

Conclusion/Discussion: LC/QTOF analysis of paroxetine positive urine samples results in observation of parent drug in human urine in spite of the earlier reports of low levels of parent drug present. Other molecules have demonstrated "efficient" ionization (e.g., fentanyl) making detection by MS/MS more likely than the relative radiolabeled abundance would predict. This may explain the observed LC/QTOF prevalence of this compound or the original radiolabeled studies may be incorrect with respect to metabolite distribution. Overall, the LC/QTOF identification of paroxetine urine metabolites is consistent with radiolabeled studies with the exception of the relative abundance of parent drug.

Keywords: Paroxetine, Urine Testing, Metabolite Distribution

P76

Fentanyl—Norfentanyl Concentrations During Transdermal Patch Application: LC-MS/MS Urine Analysis

Oneka T. Cummings[1], Jeffrey R. Enders[1], Gregory L. McIntire[1], **R. Backer*[1]**, A. Poklis[2]; [1]Ameritox, LLC., Greensboro, NC, [2]Department of Pathology, Virginia Commonwealth University, Richmond, VA

Background/Introduction: Poklis and Backer [1] published a survey of the concentrations of fentanyl and norfentanyl that could be expected in urine from patients using Duragesic®, a transdermal fentanyl patch. That study employed a relatively small number of patient data points and analysis by GC/MS. This work examines a larger population of patient positives for fentanyl and norfentanyl to determine whether more than a decade later the original report remains accurate in predicting the range and median levels of fentanyl and norfentanyl concentrations physicians can expect to see from their patients.

Objective: This work compares the original GC/MS work of Poklis and Backer [1] with historical data taken by LC-MS/MS over a period of nearly 5 years (January 2011- September 2015). The number of patient results examined exceeds 73,000 data points. Furthermore, this work details statistical methods developed to retrospectively transform and normalize historical fentanyl test results from urine drug testing to afford a near Gaussian distribution.

Method: Patient urine specimens were submitted for quantitative Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) fentanyl and norfentanyl analysis. The resulting data were used to compare with the original work of Poklis and Backer [1] and to develop the statistical distributions discussed in this presentation. The juxtaposition of the raw testing results is used to compare concentration ranges observed in these two studies while the effort to develop the statistical distribution is intended to supplement these findings and provide a "comparison" tool that can be useful to clinicians. The LOQ for this method is 2 ng/mL for fentanyl and 10 ng/mL for norfentanyl while the ULOQ is 2500 ng/mL for both analytes. The method was validated as described in Enders and McIntire [2]. The data analysis and model development were conducted using R version 3.1: A language and environment for statistical computing.

Result: Statistical comparison of the data sets supports the accuracy of the initial study at lower doses of transdermal fentanyl and norfentanyl. While statistically different at higher doses, the fentanyl results track well over the range of dose levels examined. However, norfentanyl results deviate at moderate and higher dose concentrations in a uniform manner. The reasons for these differences are not identified but may speak to the need for extraction and derivatization preceding GC/MS analysis.

Conclusion/Discussion: Comparison of a large number of fentanyl and norfentanyl results with earlier work suggests that other than extreme outliers, the data compare favorably for fentanyl [1]. Norfentanyl data are not statistically significantly different at the 50 μg/hr dose level but are higher for the LC-MS/MS results than the GC/MS results at elevated doses. Transforming and normalizing these historical urine fentanyl data results in a near Gaussian distribution that can be used to assess patient consistency with this historical population.

References:

[1] Poklis, A., and Backer, R. (2004) Urine Concentrations of Fentanyl and Norfentanyl During Application of Duragesic® Transdermal Patches, J. Anal. Toxicol. 28, 422-425.

[2] Enders, J., and McIntire, G. L. (2015) A Dilute and Shoot LC-MS Method for Quantitating Opioids in Oral Fluid, J. Anal, Toxicol, 39 (8), 662-667.

Keywords: Fentanyl, Norfentanyl, LC-MS/MS

P77

Validation of DUID WB Biochip Array for 20 Different Assays Using the Randox Evidence Investigator

Heather Dyer*[1], Stephen Pierce[1], Kim Buffum[1], Jamie Foss[1], Rachel Walls[2]; [1]Maine Health & Environmental Testing Laboratory, Augusta, ME, [2]Randox Toxicology, Crumlin, Co Antrim, UK

Background/Introduction: Immunoassay tests are widely used in the field of forensics for the screening of biological specimens. They are a reliable test for the rapid screening of drugs of abuse. The Maine Bureau of Highway Safety purchased a Randox Evidence Investigator for the Health & Environmental Testing Laboratory. The DUID WB Biochip array is a multiplex array for the semi quantitative determination of 20 drugs of abuse from one whole blood sample. The assays include: Oxycodone, Dextromethorphan, Meprobamate, Methamphetamine, Barbiturates, Benzodiazepines I & II, Methadone, Opiates, PCP, Benzoylecgonine, Zolpidem, Tricyclic Antidepressants, THC, Tramadol, Amphetamine, Fentanyl, and Buprenorphine. A method validation study was performed to evaluate the Randox DUID WB biochip array.

Objective: To evaluate the DUID WB biochip array for the use of screening whole blood samples from DUI related incidents. The method validation study was completed following SWGTOX method validation guidelines for immunoassay tests. Limit of detection, inter-assay precision, and percent recovery were evaluated.

Method: Blank whole blood was fortified with the standardizing antigens at concentrations of 50% below the cut off, at the cut off, and 50% above the cut off for the 20 analytes. Nine lyophilized calibrators and two controls included in the Randox DUID WB kit were reconstituted and analyzed with each run. Three replicates of each concentration were analyzed over 5 separate runs carried out by one analyst. Each run was completed using the method provided by the manufacturer, using 60 uL of sample diluted 1:4 in specimen diluent. Twenty replicates of blank whole blood were assessed for limit of detection, using the mean plus 3SD. An on-going correlation study is being conducted with an outside laboratory. Data verified from GC-MS/LC-MS has shown good correlation, and presently no false positives have been identified.

Result: The cut-off values for the 20 assays ranged from 2 ng/mL to 100 ng/mL. The inter-assay precision at the cut-off for all assays had a coefficient of variation ranging from 5% to 18%. The limit of detection was found to be at least 7 times below the cut off for all analytes. The percent recovery ranged from 62% to 171%.

Conclusion/Discussion: The Randox DUID WB biochip array used with the Evidence Investigator was determined to be a valid method. After evaluating limit of detection, inter-assay precision, and percent recovery, the assay proved to be reliable for the screening of multiple drugs of abuse in whole blood using a small sample volume.

Keywords: Method Validation, Immunoassay, Randox Toxicology

P78

Development of an ELISA for the Detection of 6-Monoacetylmorphine (Heroin Metabolite) in Human Urine, Blood and Oral Fluid

Tina German, **James Clarke***; Neogen Corporation, Lexington, KY

Background/Introduction: Heroin is a synthetic acetylated analog of morphine. Heroin abuse is a worldwide concern. Following administration, heroin is rapidly metabolized to 6-monoacetylmorphine (6-MAM) and more slowly to morphine. 6-MAM metabolite can be found present in urine, blood and oral fluid. 6-MAM presence is a definitive biomarker for heroin abuse, and allows for specific heroin monitoring without potential interference from typical legally prescribed opiates. Monitoring of 6-MAM can be accomplished using conventional chromatographic-based methods. More recently homogeneous immunoassays have been developed, but are better suited for urine and oral fluid screening applications due to high blood matrix opacity. Current homogeneous immunoassays targets cutoffs for 6-MAM monitoring in urine at 10 ng/mL. The newest 2015 SAMHSA proposed cutoff guidelines for 6-MAM detection in oral fluid is 3 ng/mL. A rapid Enzyme-Linked Immunosorbent Assay (ELISA) screening method that allows for direct monitoring of 6-MAM in all frequently encountered matrix types, especially whole blood at current and recommended concentrations, would be beneficial for routine testing applications.

Objective: This study reports the development of a highly specific and sensitive (ELISA) for the detection of 6-MAM in human urine, whole blood, and oral fluid samples.

Method: An antiserum was developed against a 6-MAM analog and used to develop a competitive assay. The assay was tested for sensitivity, reproducibility, cross-reactivity, and matrix interference in diluted human urine, whole blood, and oral fluid.

Result: The limit of detection of 6-MAM in assay buffer is 0.1 ng/mL. Human urine (n=80), whole blood (n=20) and oral fluid (n=80) sample populations containing no drug were tested for assay interference and found to produce no false positives at typical 10 ng/mL cutoff and 3 ng/mL proposed SAMHSA oral fluid cutoff. 6-MAM can be detected reproducibly in 1:10 diluted human urine, 1:10 diluted whole blood, and 1:4 diluted oral fluid at concentrations as low as 2.5 ng/mL, 5 ng/mL, and 1 ng/mL, respectively. Upper range of detection in 1:10 diluted human urine, 1:10 diluted whole blood, and 1:4 diluted oral fluid was 80 ng/mL, 80 ng/mL, and 40 ng/mL, respectively. Percent cross-reactivity was calculated relative to 6-MAM with levorphanol, morphine, heroin and norcodeine having the highest relative cross-reactivity at 0.51%, 0.04%, 0.03%, 0.03%, respectively. All other common opiate drugs tested were below 0.03%. The antisera did not show cross-reactivity (<0.03%) with a comprehensive selection of common non-opioid drugs and compounds. The reproducibility of the dose response curve was evaluated and found to be less than 5% at all concentrations tested.

Conclusion/Discussion: The described 6-MAM ELISA is robust, sensitive and applicable for the testing of human urine, whole blood and oral fluid. The assay is sufficiently sensitive to detect 6-MAM at current homogeneous urine assay cutoff levels as well as the SAMHSA proposed oral fluid cutoffs with potential to go significantly lower or higher if required. The ability to detect 6-MAM in whole blood without the need for extensive clean-up will benefit users in broadened screening applications. The low cross-reactivity observed with other opioid and non-opioid drugs suggests specific 6-MAM detection capability.

References:

Baselt, R.C. (2004). *Disposition of toxic drugs and chemicals in man*, 7th edition, Foster City, California: Biomedical Publications.

Moffat, A.C. Osselton, M.D., Widdop, B. (2004) *Clarke's Analysis of Drugs and Poisons*, 3rd edition, London, UK: Pharmaceutical Press.

UNODC, *World Drug Report 2012* (United Nations publication, Sales No. E.12.XI.1).

Lipari, R.N. and Hughes, A. *The NSDUH Report: Trends in Heroin Use in the United States: 2002 to 2013*. The CBHSQ Report: April 23, 2015. Substance Abuse and Mental Health Services Administration, Center for Behavioral Health Statistics and Quality. Rockville, MD.

Keywords: 6-MAM, ELISA, Forensic

P79

Generic Sample Extraction Workflow for Analysis of 76 Drugs in Urine by LDTD-MS/MS (Screening) and LC-MS/MS (Confirmation)

Alex Birsan*[1], Pierre Picard[1], Philip Dimson[2], John D. Laycock[2], Karsten Liegmann[2], David Hall[2], Jean Lacoursiere[1]; [1]Phytronix Technologies, Inc., Quebec, Canada, [2]SPEware Corporation, Baldwin Park, CA

Background/Introduction: Toxicology laboratories are looking for new ways to improve their analysis by lowering operating costs and increasing throughput all the while maintaining quality data reporting. The use of a generic sample preparation for presumptive analysis of all drug classes in urine and subsequent use of the same extract for definitive testing can provide a significant cost and time savings approach. To increase the sample throughput, Laser Diode Thermal Desorption Mass Spectrometry (LDTD-MS/MS) was used employing the generic sample extraction. Presumptive positive samples were transferred to LC-MS/MS for confirmation using the same sample extract.

Objective: LDTD-MS/MS offers specificity combined with an ultra-fast analysis approach for an unrivaled presumptive testing method. To develop this application, we focused on performing an efficient and generic extraction method for all drug polarities and concurrent use for presumptive and definitive testing method. 76 drugs are analyzed in urine from different classes (opiates, benzodiazepines, amphetamines, barbiturates, etc.) are analyzed simultaneously, with quantitative screening results obtained in less than 9 seconds per sample.

Method: The following drugs were spiked in urine at a 50%, 100% and 200% of the required concentration suggested in literature (SAMHSA guideline). Certain drugs were screened using positive ion mode: Benzodiazepine group, opiate group, amphetamine group, antidepressant group and more. Others were screened using negative ion mode: barbiturate group and THCC. Urine samples were first hydrolyzed. 50 µL of urine sample is mixed with 100 µL of internal standard/buffer/glucuronide solution, added to SPE-LDX-1 and hydrolysis performed at 55°C for 30 minutes in cartridge. The cartridge was then washed. Barbiturate group, THCC, Meprobamate and Carisoprodole drug were eluted with a mixture of Hexane:EtAc then all other drugs were eluted using basic elution solution. 5 µL of elution solution was spotted on LazWell plate for LDTD-MS/MS screening. Using the same extract, samples that gave a positive presumptive result were confirmed by LC-MS/MS using generic gradient on C18 column in ESI ionization mode.

Result: LDTD-MS/MS operated in MRM mode allows rapid measurement of all drugs desorbed simultaneously. One specific transition is used per compound for screening and two transitions are monitored for each drug in confirmation. Four internal standards (IS) are monitored in screening, although 67 more IS are available in the extraction for use in confirmation. Ionization is performed in positive/negative mode for elution one group and positive mode for the second elution group. Analysis includes spiked drugs in urine and real samples. Precision at the decision point, interference study and dilution integrity were reported for the new screening approach. All compounds give good responses around the cutoff level. Drug concentrations in real samples were also evaluated in LC-MS/MS with a long gradient to separate each drug class. The benzodiazepine, opiate and marijuana metabolites group requires β-glucuronide enzyme treatment for drug detection.

Conclusion/Discussion: A generic sample preparation allows robust drug screening in urine samples using the LDTD technology. Sample-to-sample run time of 9 seconds is achieved with the analysis of 70 drugs in positive mode and 6 drugs in negative mode. Using the same sample extract, positive presumptive samples were successfully confirmed with a LC-MS/MS method.

Keywords: LDTD-MS/MS; LC-MS/MS; Drug Screening and Confirmation

P80

One-Step Extraction Procedure for Presumptive and Definitive Urine Testing for More Than 100 Drugs - Fast Screening Using LDTD-MS/MS Method

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

Background/Introduction: Several immunoassay tests are normally required to screen different drug families in urine samples. Presumptive positive samples are then extracted and analyzed using LC-MS/MS method for definitive testing. Recently, a number of laboratories have transitioned to mass spectrometry detection to increase the sample analysis throughput and quality of presumptive testing; therefore we developed a single one-step extraction procedure for both presumptive and definitive urine testing. To maintain sample throughput equal to previous immunoassays, Laser Diode Thermal Desorption Mass Spectrometry (LDTD-MS/MS) was used for screening. Presumptive positive samples were then confirmed using LC-MS/MS method using the same sample extract.

Objective: Development of single one-step extraction approach was challenging because drug panels contain molecules with wide chemical proprieties. All drugs were extracted and screening was performed using Laser Diode Thermal Desorption Mass Spectrometry (LDTD-MS/MS) first, presumptive positive samples were confirmed using LC-MS/MS method using the same sample extract as the presumptive testing. To develop this application, we focused on performing a fast and simple extraction method. More than 100 drugs of abuse from different classes (opiates, benzodiazepines, amphetamines, barbiturates, cocaine, PCP, etc.) are analyzed simultaneously, with quantitative presumptive screening results obtained in less than 9 seconds per sample.

Method: To develop this one-step extraction procedure, different approaches and extraction techniques were evaluated. The following drugs were spiked in urine at a 12.5, 25, 50 and 100 ng/ml. The following listed drugs were screened using positive ion mode: Opiate drugs, Benzodiazepine drugs, Amphetamine drugs, Cocaine metabolites, Antidepressant drugs and more. The following drugs were screened using negative ion mode: Barbiturate drugs and THCC. Urine samples were first hydrolyzed using purified □-glucuronide to obtain the free form of drug. Four different extraction strategies were tested: i and ii) liquid-liquid extraction (LLE) in basic/acid pH, iii) cartridge with SCX packing and ix) cartridge with RP/WAX packing. Extraction conditions were set to obtain good drug signal and minimize the extraction of uric acid and acetaminophen. After purification, extract was spotted in LazWell. Analysis was performed in LDTD-MS/MS for screening and the same extract used in LC-MS/MS for confirmation.

Result: For presumptive testing, LDTD-MS/MS operated in MRM mode, allows ultra-fast measurement of all drugs desorbed simultaneously. Specific transitions are monitored for each drug to quantitate calibrator levels. Ionization is performed in positive mode for group one and negative mode for the second group. The acidic LLE allows for a good extraction of drug analyzed in negative mode at very low cut-off level. The LLE basic gave a good extraction of basic drug but was not generic enough for all compounds. The SCX cartridge is a good generic approach for all drugs analyzed in positive mode to reach low concentration cut-off but no recovery for drugs analyzed in negative mode. The RP/WAX cartridge allows us to extract all drugs screened in positive and negative mode and the extract was adapted to minimize the high level of acetaminophen found in some real patient samples. For all extractions, the cut-offs are attained with the LDTD-MS/MS. Precision at the decision point, limit of detection, interference study and dilution integrity were reported for the new screening approach. A generic LC-MSMS gradient with ESI ionization were used as confirmation test method.

Conclusion/Discussion: The LDTD technology combined with a generic sample preparation allows robust drug screening in urine samples. Using the same sample extract, definitive testing is performed with LC-MS/MS method without additional re-extraction of samples.

Keywords: LDTD-MS/MS, Drug Screening

P81

Method Validation of Nicotine and Metabolites by HPLC-MS/MS Reveals Low Clinical Utility for Tobacco Alkaloid Anabasine

Matthew Feldhammer[1], **Dan Menasco*[2],** Willa Zhang[1], James C. Ritchie[1]; [1]Department of Pathology and Laboratory Medicine, Emory University School of Medicine, [2]Biotage AB, Charlotte, NC

Background/Introduction: Tobacco exposure is strongly correlated with increased postoperative risks for infections as well as negative outcomes in both transplant donors as well as recipients. Nicotine screening has become a routine part of the clinical workup for pre-transplant eligibility as well as many elective surgical procedures. Routine screening protocols often rely on EMIT based assays, which lack the capability to distinguish nicotine from some of its longer lived metabolites such as trans-3'-hydroxycotinine. Additionally, these screens also fail to distinguish between active tobacco use and Nicotine-Replacement-Therapy (NRT) by monitoring anabasine, leaving some patients with false positive results potentially making them ineligible for organ transplantation and various surgeries. The clinical utilization of LC-MS/MS technology can bypass this limitation by simultaneously providing quantitative analyses of nicotine as well as several of the metabolic products and an alternate alkaloid anabasine, which is purported to distinguish patients using NRTs, respectively.

Objective: In an effort to overcome this hurdle several manufacturers have begun implementing automated sample extraction stations to reduce the workload and improve sample turn-around time. We therefore set out to validate an LC-MS/MS method for the quantification of nicotine, its metabolites: cotinine, trans-3'-hydroxycotinine, and the tobacco plant alkaloid anabasine in patient urine who's smoking status was self-reported to Emory clinicians. All four metabolites will be examined under routine clinical screening applications using the Biotage® Extrahera™ liquid handling station to evaluate the utility of sample prep automation.

Method: Patient urine samples were obtained from our orthopedics and transplant centers. Blank urine analyzed by automation were purchased from UTAK. Interfering matrix components were removed using Biotage® supported liquid extraction (SLE) columns either by vacuum manifold or on the Extrahera™ (Biotage®) automation station. Nicotine, cotinine, trans-3'-hydroxycotinine and anabasine were chromatographically resolved over 7 minutes on a Waters 50 x 2.1, 1.7 □m BEH-C_{18} column and monitored by MRM at 163.2→130.1, 177.2→80.0, 193.1→133.9, and 163.0→94.3, respectively, using a Waters Xevo TQ-S mass-spectrometer. Linear responses were evaluated within a range of 5-5000 ng/mL. Patients smoking status was self-reported to Emory clinicians and obtained by analyses of the electronic medical record. NRT users (self-defined) were grouped into the non-smoking category unless there was evidence of anabasine present (only the case for 1 patient). Patients were categorized as either active smokers (nicotine > 30 ng/mL; cotinine > 50 ng/mL; trans-3'-hydroxycotinine > 120 ng/mL; anabasine > 3 ng/mL) or non-smokers if they did not fulfill the above criteria.

Result: The quantification limits of the method were 1-5 ng/mL and the limits of detection ranged from 0.6-5 ng/mL. The linear range for all analytes was confirmed over 5-5000 ng/mL. Interday and intraday precisions for all analytes had a C.V. of <15% at the low end of the quantification limit. Method comparison studies were performed with either GC-MS (within institution method) or by LC-MS/MS (ARUP) correlation coefficients were determined to be between (0.95-0.99). Under the auspice of automation, recoveries were determined to be 107, 95, 53, and 107 percent for nicotine, anabasine, trans-3'-hydroxycotinine, and cotinine, respectively. Moreover, sample processing times (completed in batches of 24) decreased by 50% using the Extrahera™ automation station when compared to manual extractions. Most surprisingly we determined that 64% of our patients that self-declared as active smokers and tested positive for nicotine had anabasine levels below <3 ng/ml.

Conclusion/Discussion: Nicotine, cotinine, trans-3'-hydroxycotinine and anabasine can be simultaneously accurately quantified in human urine by LC-MS/MS to distinguish between smokers and non-smokers. Moreover, workflow efficacy improved significantly when automation was integrated into the pipeline. Interestingly the clinical utility of anabasine as a means of detecting active tobacco use is highly questionable, as up to 64% of admitted smokers had no detectable levels of the alkaloid.

Keywords: Nicotine, Anabasine, Nicotine-Replacement-Therapy

P82

Comparison of the Concentrations of Cocaine/Benzoylecgnonine/Cocaethylene (COC/BE/CE) in the Blood of Driving and Postmortem Cases

Albert A. Elian*[1], Jeffery Hackett[2]; [1]Massachusetts State Police Crime Laboratory, Maynard, MA, [2]Dovecot, Liverpool, Merseyside, UK

Background/Introduction: The testing of COC/BE/CE in antemortem (AM) and postmortem (PM) blood samples are routinely performed by forensic toxicology laboratories. In this study, samples from both types of cases are presented having been analyzed by the same extraction method (solid phase extraction (SPE)) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) in AM cases and gas- chromatography- mass spectrometry (GC-MS) for PM cases.

Objective: This study was initiated with the idea of comparing the concentrations of COC/BE/CE in postmortem blood samples with those found in drugs/driving cases when extracted and analyzed using LC-MS/MS and GC-MS. The data will assist those analysts involved in the interpretation of cocaine and metabolites in both AM and PM types of cases.

Method: Samples of blood (1 mL (Calibrators, controls, and test)) containing deuterated internal standards were diluted with 4 mL of phosphate buffer (pH 6), after which the samples were extracted on mixed mode SPE columns. The SPE columns having being pre-conditioned with methanol, deionized (DI H_20) water and 0.1M phosphate buffer (3 mL, 3 mL, 1 mL respectively) prior to sample loading. The SPE cartridges (Clean Screen®DAU 206 (UCT Inc., Bristol PA)) were washed with DI H_2O, 1.0 M aqueous acetic acid, methanol (3mL of each) and dried. Each SPE column was eluted with 3 mL of a solution of methylene chloride-isopropanol-ammonium hydroxide (78-20-2). The samples were evaporated to dryness under nitrogen at 40 ºC, and the residues dissolved in 100 µL of mobile phase (0.1% aqueous formic acid/ acetonitrile containing 0.1% formic acid (95:5)) for LC-MS/MS analysis. LC-MS/MS was performed in positive multiple reaction monitoring (MRM) mode (details presented). For GC-MS the dry SPE residues were derivatized with 50 µL of 1:1 Ethyl acetate-BSTFA (1%TMCS). GC-MS analysis was performed in electron ionization mode (details presented).

Result: The limits of detection/ quantification for this method were determined to be 5.0 ng/ mL and 10.0 ng/ mL by GC-MS and LC-MS/MS, respectively for the analytes (COC, BE, CE). The method was found to be linear from 10 ng/ mg to 1000 ng/ mL (r^2>0.999). Recoveries were found to be greater than 95%. Interday and Intraday analysis of the compounds were found to < 8% and < 10 %, respectively. Matrix effects were < 6%. The results of postmortem samples involving 80 PM males (mean age= 48 yrs): the mean values (ng/ mL) were: 790 (range 0 to 1570) (COC), 1670 (range 65 to 2000) (BE), 83 (range 0 to 480) (CE), respectively; for 60 PM females (mean age = 32 yrs): the mean values (ng/ mL) were: 680 (range 0 to 1420) (COC), 1230 (range 50 to 1550) (BE), 65 (range 0 to 390) (CE), respectively. With drugs/driving cases: For 40 AM male cases (mean age= 28 yrs): the mean values (ng/ mL) were: 530 (range 0 to 1330) (COC), 900 (range 40 to 1100) (BE), 54 (range 0 to 390) (CE), respectively; in 30 AM females (mean age= 26 yrs): the mean values (ng/ mL) were: 450 (range 0 to 1280) (COC), 970 (range 32 to 1770) (BE), 45 (range 0 to 230) (CE), respectively.

Conclusion/Discussion: This study shows that using a single extraction method and the use of GC-MS and LC-MS/MS instruments for the analyses, cocaine and its metabolites from AM and PM cases can be evaluated. The data indicates that although the parent compound (COC) is of a similar magnitude in AM and PM cases, the concentrations of the primary metabolite (BE) is higher in PM cases than AM ones. This may be due to degradation of the COC while in the body. The concentrations of CE are also of similar magnitudes indicating the prevalence of ethanol and cocaine ingestion occurring in these types of cases. The interpretation of concentrations of cocaine/metabolites in postmortem samples relative to those obtained from living subjects should be evaluated based not only on toxicology but case and medical history too.

Keywords: Cocaine, SPE, Blood

P83
Stability of Ethanol in Oral Fluid Collected with the Quantisal® Device

James Tuyay, Joshua A. Chou, **Cynthia Coulter*,** Christine Moore; Immunalysis Corporation, Pomona, CA

Introduction/Objective: Analyze, by GC-FID, authentic Quantisal® collected oral fluid samples for alcohol at different time points from 1 month to 12 months to determine stability of ethanol in Quantisal® buffer.

Method: A recovery study was performed on the Quantisal® collection device to determine the efficiency of recovering ethanol from the collector. Spiked replicates (n=6) were prepared at 20 and 80mg/dL. Recovery was determined by comparing concentrations of ethanol directly added to oral fluid to ethanol concentrations recovered from pad collected spiked oral fluid. Percent recovery for the 20 and 80mg/dL spikes were calculated at 101 and 97% respectively. Oral fluid samples routinely received for ethanol analysis were re-analyzed at 1, 2, 4, 6, and 12 months. Samples were transferred from the original device and stored in borosilicate glass tubes at 4°C. Samples were screened for alcohol and drugs of abuse upon receipt. Positive samples were analyzed by GC-FID for ethanol. All samples (N=11) and calibrators were diluted 1+3 with Quantisal® buffer prior to analysis.

Result: Original ethanol concentration of samples ranged from 21 to 96mg/dL. After 12 months samples showed no significant loss of ethanol with the greatest loss at 10% after 12 months. Two samples stored for 2 months had losses of 27 and 25% and one sample stored for 12 months had a loss of 100%. All three samples appeared to have bacterial growth prior to the retest.

Data:

Sample ID	*Original (mg/dL)*	*Retest (mg/dL)*	*% Recovery*	*% Loss*	*Storage*	*Notes*
1	21	22	104.8	0	12 month	
2	65	58	89.2	10	12 month	
3	96	0	0.0	100	12 month	Bacterial Growth
4	27	27	100.0	0	6 month	
5	84	84	100.0	0	6 month	
6	30	31	103.3	0	4 month	
7	67	67	100.0	0	4 month	
8	79	57	72.2	27	2 month	Bacterial Growth
9	57	43	75.4	24	2 month	Bacterial Growth
10	57	59	103.5	0	1 month	
11	58	60	103.4	0	1 month	

Conclusion/Discussion: Ethanol oral fluid samples collected with the Quantisal® device and stored in borosilicate tubes at 4°C are stable for up to 12 months assuming there is no bacterial growth within the sample. To help ensure reproducible results proper collection technique must be used to limit the amount of foreign material in the sample that could potentially cause bacterial growth.

Keywords: Alcohol, Oral Fluid, Stability

P84

An Efficient, Robust Method for the Determination of Cannabinoids in Whole Blood by LC-MSMS

Nicholas B. Tiscione*[1], Russell Miller[1], Xiaoqin Shan[1], Jessica Sprague[2], Dustin Tate Yeatman[1]; [1]Palm Beach County Sheriff's Office, West Palm Beach, FL, [2]University of Central Florida, Orlando, FL

Background/Introduction: Due to the prevalence of cannabinoids in forensic toxicology casework it is desirable to have an efficient cannabinoid analytical method that uses a small volume of blood and requires minimal sample preparation. Methods reported in the literature are often labor intensive, require special sample preparation materials, use 1 mL or more of specimen, or are difficult to replicate.

Objective: The objective was to identify a method using liquid chromatography with tandem mass spectrometry (LC-MSMS) for the analysis of cannabinoids in whole blood and conduct method validation studies to provide a simple, rapid sample preparation that was cost-effective and used minimal sample volume, consumables, and reagents.

Method: The quantitation of cannabinoids in whole blood method using liquid-liquid extraction was validated by evaluating selectivity/specificity, ionization suppression or enhancement, the calibration model, sensitivity, bias, precision, reportable range, carryover, extract stability, ruggedness/robustness, a case sample comparison, and an estimation of uncertainty of measurement. The general validation scheme was based on those described previously (1-3) and has been used to validate several methods within the Toxicology Unit at the Palm Beach County Sheriff's Office. All instrumental and data analysis parameters were determined prior to the start of validation as part of method development and optimization. Deuterated internal standards were used for each target analyte.

Result: The validated limit of detection and limit of quantitation using 0.5mL of whole blood were 1 ng/mL for delta-9-tetrahydrocannabinol (THC) and 11-hydroxy-delta-9-tetrahydrocannabinol (OH-THC) and 5 ng/mL for 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THCA). Each analyte demonstrated a zero-order linear range ($r^2 > 0.990$) with 1/x weighting of 1-40 ng/mL for THC and OH-THC and 5-200 ng/mL for THCA. The CV of replicate analyses was within 14%. Bias was within ± 13% of the prepared concentration. There was no matrix interference from 10 different whole blood sources that did not contain the target analytes. There was no interference from 75 related compounds that are commonly identified in whole blood case samples.
The laboratory's existing blood THC quantitation procedure used protein precipitation of 1 mL of whole blood followed by solid phase extraction and derivatization to form TMS derivatives of THC and THCA and the corresponding deuterated internal standards for each (OH-THC was not included). Analysis was by gas chromatography mass spectrometry (GC-MS) with selected ion monitoring. The linear calibration range was from 2.5 to 100 ng/mL with an LOD of 1 ng/mL and an LOQ of 2.5 ng/mL for THC and THCA. The case comparison that was conducted yielded comparable results. The analysis run time per sample was 60 minutes by GC-MS and 9 minutes by LC-MSMS. The LC-MSMS method provided a simple, rapid, and lower cost sample preparation with drastically shorter instrument analysis time that required half the sample volume and yielded better sensitivity for THC.

Conclusion/Discussion: The robust procedure for the quantitation of cannabinoids from whole blood, using LC-MSMS, provides a sensitive, efficient method that employs a rapid and simple liquid-liquid extraction, uses 0.5 mL of specimen, and has been successfully implemented on casework evidence in two forensic laboratories.

Keywords: Validation, Cannabinoids, LC-MSMS

P85

Evaluation of Drugs of Abuse and Pesticides from Liver Matrix Following Homogenization and Supported Liquid Extraction (SLE) Prior to GC/MS Screening

Rhys Jones[1], Lee Williams[1], **Adam Senior***[1], Alan Edgington[1], Helen Lodder[1], Geoff Davies[1], Steve Jordan[1], Claire Desbrow[1], Paul Roberts[1], Dan Menasco[2], Elena Gairloch[2]; [1]Biotage GB Limited, Distribution Way, Dyffryn Business Park, Cardiff, UK, [2]Biotage, Charlotte, NC

Background/Introduction: In postmortem cases, where drugs or pesticides have been used for their poisonous properties, traditional matrices such as urine and whole blood may be inappropriate for qualitative and quantitative analysis. As the site of metabolism for most drugs and toxins, the liver may provide more insight to cause of death than other bodily fluids. Screening drugs of abuse can be complicated due to the wide variation of functional groups associated with different analyte classes. Most extraction techniques cannot extract all analytes using a single procedure without using non-optimal extraction protocols resulting in compromised extract cleanliness. Supported liquid extraction is a 96-well plate or individual column based extraction technique analogous to traditional liquid-liquid extraction. The technique allows for the simultaneous analysis of cross functional analytes in a single extraction protocol without forfeiting extract cleanliness.

Objective: The objective was to develop a common extraction procedure for multiple drug panels from liver tissue matrix using supported liquid extraction (SLE) prior to GC/MS analysis. The drug suites were amphetamines, barbiturates, benzodiazepines, cocaine, ketamine, THC and pesticides.

Method: Pig liver specimens (100-500mg) were homogenized using a BeadRuptor 24 unit. Efficient homogenization was achieved using spherical, ceramic beads with high speed rotation. Typically 200 mg of matrix was homogenized with 1.8 mL of aqueous organic mixtures. Optimization of extraction was performed by spiking analytes at 1000 ppb from a broad range of panels into liver/solvent homogenate. Deuterated internal standards (D_3-D_5) were used for Amphetamine, Diazepam, and THC.

Extraction conditions were evaluated using spiked liver homogenate at various pH values with aqueous organic solvent mixtures. Sample load volumes on ISOLUTE SLE+ 1 mL capacity columns, from 500 µL to 1 mL were evaluated for optimal extract cleanliness. Analyte extraction was evaluated using multiple aliquots of water-immiscible solvents MTBE, DCM, hexane and EtOAc at volumes of 2 to 3 mL into glass culture tubes. Evaporation was performed with air at ambient room temperature.

Derivatization was optimized to a single approach for all analyte panels. Some had no requirement for derivatization but were not negatively affected by the silyation agent. Upon dryness, 25 µL EtOAc and 25 µL N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) was added, vortex-mixed and transferred to glass vials prior to heating at 80 °C on a heat block for 30 minutes. GC/MS analysis was performed injecting 1 µL in split mode. All samples were analyzed using an Agilent 7890 GC with a 5975 MSD. Chromatography was performed on an Agilent J&W DB-5 capillary column; 30 m x 0.25 mm ID x 0.25 µm using 1.2 mL/min helium. Positive ions were acquired using electron ionization operated in SIM mode.

Result: Multiple protocols were evaluated and the optimal recoveries were observed following loading 500 µL of liver homogenate in a mixture of 50/50 water/methanol at non-adjusted pH, and elution with DCM. All drug and pesticide panels delivered recoveries were greater than 70% with RSDs below 10%. Increasing the pH value of the sample resulted in decreased cleanliness and loss of recovery of some analytes.

Calibration lines were constructed with spiked homogenates from 50-2500 ppb. Limits of quantitation based on signal to noise ratios greater than 10:1 were deemed to be between 50-500 ppb. Full details will be presented in the poster.

Conclusion/Discussion: This poster describes the suitability of ISOLUTE SLE+ for the rapid and reliable extraction of multiple analyte panels from liver tissue in a single assay prior to GC/MS screening, and ultimately its applicability for the use in post mortem cases.

Keywords: SLE+ (Supported Liquid Extraction), Liver, GC/MS

P86

Evaluation of Novel Automated Sample Preparation Compared to Manual Processing in Forensic Toxicology

Rhys Jones[1], Lee Williams[1], Katie-Jo Teehan[1], Adam Senior[1], Alan Edgington[1], Helen Lodder[1], Geoff Davies[1], Steve Jordan[1], Claire Desbrow[1], **Paul Roberts***[1], Dan Menasco[2], Elena Gairloch[2]; [1]Biotage GB Limited, Distribution Way, Dyffryn Business Park, Cardiff, UK, [2]Biotage, Charlotte, NC

Background/Introduction: Data reliability in forensic toxicology is of paramount importance. The possibility of false positives and/or false negatives can have wide ranging consequences and impacts decisions on various parts of the analytical method development process.

Objective: This poster compares the performance of manual processing to a novel automated sample preparation system prior to GC/MS or LC-MS/MS analysis. Emphasis will also be placed on the potential for 96-well cross contamination and strategies for its elimination.

Method: Extraction was performed using ISOLUTE® SLE+ 400 µL fixed well plates or 1 mL capacity columns. Manual processing was conducted using a positive pressure manifold for both 96-well plates, PPM-96, and 48 columns, PPM-48. Automated sample preparation was performed using the Biotage® Extrahera™ system configured with either a 24 or 96 positive pressure head for column or 96-well plate processing, respectively. Samples were analyzed using either GC/MS or LC-MS/MS. An Agilent 7890 GC with a 5975 MSD acquired positive ions using electron ionization operated in the single ion monitoring (SIM) mode. LC-MS/MS analysis was performed using Waters ACQUITY UPLC coupled to either a Premier XE or XEVO TQS triple quadrupole mass spectrometers.

Result: Supported liquid extraction protocols were previously developed for multiple drugs of abuse classes from urine, oral fluid and whole blood matrices. Volumes were optimized for both 96-well plate and column processing depending on application area and required limits of quantitation. Acceptable recoveries > 70 % with corresponding RSDs below 10 % were achieved for all drugs of abuse panels. Optimized extraction protocols were transferred to the Extrahera sample preparation platform. Solvent and sample pipetting, sample mixing and positive pressure processing to affect precise flow control were optimized for each matrix. Good correlation was observed between manual and automated processing while the Extrahera generally provided better precision and accuracy. Calibration curves demonstrated excellent linearity and coefficients of determination, r^2 >0.99 for all analytes. Cross contamination was investigated for the 96-well plate format. Dye experiments demonstrated the potential for issues at various stages during manual processing. Optimized settings using the Extrahera did not show any cross contamination. Sample evaporation cross contamination was most difficult to eliminate. The use of a novel anti-cross talk plate mat was used to eliminate evaporative issues proved using dyes and amphetamines as the target analytes.

Conclusion/Discussion: This poster describes effective use of a novel sample preparation system for both column and 96-well plate format and strategies for the elimination of cross contamination.

Keywords: Automated Sample Preparation, Cross Contamination, Supported Liquid Extraction

P87

The Best of Both Worlds: LC-QTOF-MS as a Method to Detect a Targeted List of 35 Drugs and Metabolites in Urine with Retrospective Data Mining Capabilities

Natalie N. Rasmussen*[1], Adam Barker[1,2], Frederick G. Strathmann[1,3]; [1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]ARUP Laboratories, Salt Lake City, UT, [3]Department of Pathology, University of Utah, Salt Lake City, UT

Background/Introduction: Compliance testing continues to be an essential component of the drug testing market. Patients in pain management programs, prescribed drugs with a high potential for addiction and abuse, often have to submit to testing to verify compliance. As an alternative to conventional immunoassay screens with reflex to confirmation, we developed a dual identification strategy with one injection. Making use of the quadrupole-time-of-flight mass spectrometer's (QTOF) quadrupole, we combined fragmentation pattern with accurate mass, retention time, and isotope fidelity to identify analytes of interest with more accuracy than previously-published LC-TOF-MS or LC-MS/MS methods.

Objective:
1. Increase confidence in results by fragmentation and comparison to a spectral library, offsetting sup-optimal scores due to uncharacterized chromatographic interferences when tested by LC-TOF-MS.
2. Ensure qualitative agreement between this method and the validated LC-TOF-MS method.

Method: QC materials in matrix and patient samples were aliquotted into a collection plate, with 180 µL of $NaHCO_3$ buffer containing seven deuterated markers, 34 µL resorufin glucuronide solution, and 16 µL of β-glucuronidase (IMCS, Columbia, SC, USA). Post-15 minute incubation, 300 µL was transferred to a Biotage Isolute SLE+ 96-well plate (Charlotte, NC, USA) for further purification. Positive pressure was applied and the sample was allowed to absorb for 5 minutes. Samples were eluted with 0.8 mL ethyl acetate and dried under nitrogen, then resuspended using a 90:10 blend of mobile phases. Samples were analyzed using an Agilent 1290 UPLC coupled to an Agilent 6550 QTOF (Santa Clara, CA, USA) operated in Auto MS/MS mode with a preferred ion list. Chromatographic separation was achieved on an Agilent Poroshell EC-C18 2.1x50mm column heated to 50^0C. Mobile phases were 5mM ammonium formate in water and Optima grade methanol. (ThermoFisher Scientific,Waltham, MA, USA) at 0.5 mL/minute. Mobile phase B ramped from 5% to 95% over 3 minutes. Data were analyzed using MassHunter Qualitative Analysis software find by formula and library search functions. Acceptance criteria include a match to retention time, isotope spacing and abundance, accurate mass, and fragmentation pattern.

Result: De-identified samples (n=160), previously analyzed by TOF were re-analyzed on the QTOF. Resorufin glucuronide acts as a hydrolysis control. The QTOF results had excellent correlation with the TOF results. Most discrepancies (i.e. finding additional metabolites) were due to the higher sensitivity of the QTOF instrument. Nine samples required confirmation by LC-MS/MS based on original TOF results due to unusual drug metabolite pattern or suboptimal quality metrics. In contrast, the QTOF was able to identify and obtain matching fragmentation spectra for all of these analytes. The QTOF missed the parent drug five times due to detector saturation at high concentrations which caused the mass to shift out of the detection window; however, metabolites of the analyte were detected. In 4 cases zolpidem and 7-aminoclonazeapm were detected by QTOF that were not reported originally because they were below the TOF assay cutoff. In a separate experiment, samples having only 6-monoacetyl morphine but no morphine, an atypical pattern in urine, were run. Collected data were searched retrospectively for heroin and retention time was verified by running a standard.

Conclusion/Discussion: The presented method is highly specific and sensitive, providing a fast, accurate way of identifying analytes. It eliminates the need for LC-MS/MS confirmation of suboptimal results because of the ability to fragment and match to a library. The method uses the ion transmission abilities of a TOF with the enhanced specificity of high-mass-accuracy detection of fragments, providing better specificity in comparison to conventional LC-TOF-MS and LC-MS/MS methods. This method can be applied to a targeted screen, while the simultaneous collection of TOF data provides a mechanism for retrospective data analysis.

Keywords: LC-QTOF-MS, Retrospective Analysis, Compliance Testing

P88

An Improved Fully Validated LC-MS/MS High Throughput Method for the Determination of Phosphatidylethanol in Dried Blood Spots Using SelexION™ Technology Coupled with a Sciex QTRAP® 6500+

Mary Jones*[1], Irene Shu[1], Leo Wang[2], Adam Negrusz[1], Joseph Jones[1], Douglas Lewis[1]; [1]US Drug Testing Labs, Des Plaines, IL, [2]SCIEX, Redwood City, CA

Background/Introduction: Clinicians and researchers have a need for an objective test to detect ethanol ingestion. Phosphatidylethanol (PEth) is an abnormal phospholipid formed in the red blood cells after excessive alcohol intake with a half-life of approximately four days. It has thus been considered an alcohol biomarker to evaluate drinking behavior in a 3-week period with a cutoff of 20 ng/mL. However, lower quantitation limit may be warranted for implications such as mortality of HIV/HCV co-infected individuals exposed to alcohol and prenatal alcohol exposure. Due to chromatographic interferences from endogenous blood matrix, lowering quantitation limit with minimal sample preparation is an analytical challenge. The separation power of the SelexION™ coupled with the use of the appropriate modifier may overcome such challenge.

Objective: To improve the sensitivity of a high throughput liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the detection of PEth in dried blood spots (DBS) with minimal sample preparation.

Method: Authentic blood samples were collected on Whatman® 903 DBS paper, and three 3-mm punches of DBS were aliquoted for sample preparation and PEth 18:1/16:0 quantitation. After addition of deuterated PEth internal standard (d31-PEth, Avanti Polar Lipids) and 1 mL of HPLC grade methanol, the DBS samples were incubated at room temperature for 1 hour. The methanol extract was then evaporated under nitrogen at 40º C, and the residue was reconstituted in 1 mL of mobile phase. The LC was an Eksigent Micro-LC 200 Plus with Halo C18 column at flow rate of 50 µL/min. Mobile phase A was 50% 2mM ammonium acetate/25% acetonitrile/25% isopropanol, and mobile phase B was 40% isopropanol/60% acetonitrile. The mobile phase B% was linearly increased from 30% to 98% in 1.5 minutes, followed by 1.5 minutes of rinse and 0.4 minutes of equilibration time. PEth was detected using a Sciex 6500+ QTRAP® with SelexION™. The modifier used was 1-propanol with compensation voltage (COV) at -1.8V and separation voltage (SV) at 3600V. The MRM transitions detected with negative ion mode were 701→255 and 701 →281 for PEth and 732→286 and 732→281 m/z for d31-PEth. The single calibrator of 20 ng/mL was made in two approaches and evaluated for equivalence by testing 102 whole blood or DBS samples: 1) PEth 18:1/16:0 (Enzo Life Sciences) spiking solution directly added to three 3-mm punches of blank Whatman® 903 paper; and 2) PEth 18:1/16:0 solution made in whole blood collected in gray top tube then spotted at 40 □L on blank DBS paper.

Result: One-hundred-and-two authentic whole blood or DBS samples were quantitated by a single-calibrator of 20 ng/mL prepared with the two approaches resulted with equivalent quantitation ($R^2 = 0.97$, $y = 0.97x - 0.46$). It was thus determined that controls and multi-point calibrators to be prepared using the first approach to validate the method following SWGTOX guidelines. The calibration model was established 5 – 200 ng/mL, and both 20 and 8 ng/mL were proven acceptable single-calibrators to quantitate PEth 18:1/16:0 within the range. The method was precise (between run and within run CV <12.4%) and accurate (bias +5 – +12.5% for controls at 25 ng/mL, 80ng/mL, and 160 ng/mL). LOD and LOQ study was performed by fortifying decreasing amount of PEth 18:1/16:0 into negative blood extracted from the DBS paper and quantitating using 8 ng/mL calibrator. The new SelexION™ coupled Sciex 6500+ QTRAP® instrument allowed us to reach LOD and LOQ of 3.2 ng/mL with satisfactory separation from endogenous blood matrix. Matrix effect showed ion suppression for both analyte and internal standard by 30.6 – 41.2%.

Conclusion/Discussion: Without any additional sample preparation steps, the PEth sensitivity was improved due to the decrease in chromatographic interference by ion mobility separation of SelexION™.

Keywords: Phosphatidylethanol, Dried Blood Spots, SelexION

P89
I'll Huff 'til I Pass Out: Cases Involving 1, 1-Difluoroethane

Theresa M. Hippolyte*, Diego Ramirez, Dakota Lima, Curtis Cruz, Jeff Walterscheid; Division of Forensic Toxicology, Armed Forces Medical Examiner System, Dover AFB, DE

Background/Introduction: This presentation emphasizes the need for vigilance when screening for alcohol and other volatiles by headspace gas chromatography. 1,1-difluoroethane (DFE) is a fluorinated hydrocarbon also known as HFC-152a or Freon 152a that is commonly used as a propellant in commercial electronic device cleaning products, and as a refrigerant. Due to its ready availability and relatively low cost, it continues to be abused either by direct inhalation or "huffed" from a bag, causing a sense of euphoria and a loss of muscle coordination. Huffing has been termed "sudden sniffing death syndrome" because it can lead to sudden death through cardiac arrhythmias.

Objective: To identify unknown peaks that are present during a volatile screen using a targeted confirmatory method for special volatiles. In most cases, scene information is instrumental in helping laboratories target for inhalant use. If scene evidence is not present then volatile screening is the first line of defense.

Method: If an unknown peak elutes early during the screening of volatiles in a blood, urine, and/or vitreous humor specimen on headspace gas chromatography with flame ionization detection, a method for the identification of special volatile compounds is used for confirmation. This analysis is performed using an Agilent 7890B gas chromatograph equipped with an Agilent 7697A headspace sampler and an Rtx1, 60 m x 0.32 mm x 3.00 µm column connected with an Agilent 5977A MSD. 250 µL of specimen and 2500 µL of a 2 mg/dL internal standard methyl-ethyl-ketone (MEK) are pipetted into a headspace vial that is sealed and incubated for 15 minutes. After an aliquot of the vapor is injected on the instrument, the method scans for a panel of volatile gases intended to cover a large number of commercial and household products.

Result: Since 2012, twenty-one cases were reported to contain DFE. Thirteen of the cases were DWI and "fit for duty" failure investigations, while the other eight were postmortem cases. DFE alone was detected in fifteen of the cases. The remaining six cases contained ethanol, oxycodone, or marijuana in addition to the DFE. Of the twenty one cases, eighteen were male (12 Caucasian between 19-24 years of age, one African-American, and five of unidentified ethnicity) and three were Caucasian females. In ten cases, the subjects were found to possess one or more canisters containing DFE, with three found to be actively engaged in huffing. Targeted analysis for special volatiles was performed on these ten cases with scene evidence. The other eleven cases without scene context were processed using routine volatiles screening. An unknown chromatographic peak was observed and confirmed using a method for identifying special volatile compounds.

Conclusion/Discussion: DFE, like NPSs, should be monitored because of their potential for recreational abuse. Most laboratories do not routinely screen for this type of drug so it is important that unknown peaks on a volatile screen/confirmation be investigated. A volatile screen should also be employed if ethanol is screened by enzymatic methods. Eleven of the cases containing DFE would have been missed if a volatile screen had not been conducted because of the lack of scene evidence. This type of finding can significantly impact a forensic toxicology investigation.

Keywords: 1, 1-Difluoroethane, Huffing, Inhalants

P90

Forensic Technology Center of Excellence Cornucopia: Informing Advancement of Emerging Technologies in Toxicology

Nichole D. Bynum*, Katherine N. Moore, Megan Grabenauer, Jeri D. Ropero-Miller; RTI International, Research Triangle Park, NC

Background/Introduction: The National Institute of Justice's (NIJ) Forensic Technology Center of Excellence (FTCoE) at RTI International manages the testing and evaluation of emerging technologies applicable to forensic science. By identifying and removing the potential barriers that often derail the implementation and acceptance of new and innovative technologies, the FTCoE places promising technical innovations in the hands of forward-thinking practitioners, stakeholders, and policy makers. This is achieved through technical evaluations, technical assistance, landscape studies and guidance resources.

Objective: The objective of this presentation is to provide the community with an overview of the FTCoE and its role in advancing emerging technologies in toxicology. This presentation provides the audience with an overview of a selection of projects conducted by the FTCoE including a breath alcohol landscape study, evaluation of an oral fluid point of care testing device for drugs of abuse, evaluation of a portable mass spectrometer for the identification of seized drugs, cheminformatic database support and knowledge transfer workshops and events that benefit the toxicology community and its stakeholders.

Method/Results: A brief description of the FTCoE's highlighted projects follow. Results from an online survey indicate that the primary barrier to broader adoption of handheld breath alcohol instruments for evidential purposes is concern over the reliability and/or defensibility of the data acquired. The FTCoE is composing a landscape report of commercially available conforming handheld breath alcohol instruments to address this issue. The goal of the landscape report is to provide decision makers and end users with exemplary situations that illustrate successful adoption, considerations for the implementation of handheld breath alcohol instrumentation, and a comparison of the current capability of commercially available breath alcohol instruments. Similarly, the FTCoE is evaluating a new handheld oral fluid device for the detection of drugs of abuse and comparison of confirmation concentrations of oral fluid and blood. An evaluation of this kind provides the community with information on the reliability of results from a point of care testing device, based on the consistency of those results with laboratory based confirmations of oral fluid and blood taken at the same time. The evaluation consists of a laboratory evaluation and a field-based environment evaluation. Assessments include performance characteristics, ease of use, and cost effectiveness. The FTCoE recently conducted an independent evaluation of the utility of portable mass spectrometry with ambient ionization sources with a focus on paper spray ionization for the rapid screening of drugs of abuse. Results from the assessment include non-technical factors such as ease of instrument operation in the field and sample preparation, as well as technical factors such as reproducibility and carryover. The FTCoE is also working to enhance the visibility of cheminformatics data by distributing ForensicDB to a wider audience and reducing duplicity of multiple database efforts. In order to enhance knowledge about new and innovative forensic technologies, the FTCoE hosted a technology transition workshop for criminal justice professionals focusing on how new technologies should be supported in order to pass a Frye or Daubert test. The FTCoE also hosted a technology transition workshop on the topic of statistics and applied mathematics in forensic science analysis. This provided attendees with information on how to apply statistics and probability theory to different areas of forensic science such as toxicology and drug analysis. All of these FTCoE activities occurred over the past year.

Conclusion/Discussion: Attendees will gain an understanding of the scope of projects the FTCoE conducts and its facilitation in technology advancement in forensic toxicology. They will understand that the FTCoE provides educational events, technical assistance and community resources, and comprehensive reports designed to inform and guide the forensic community on relevant topics originating from credible research and expertise. By providing these essential resources, the FTCoE promotes changes in forensic science policy and procedure, which improves criminal justice and public safety practices on a national scale.

Keywords: Technology Advancement, Emerging Technologies, Technology Transfer

P91
Testing for Interfering Substances Using OIML Recommended Concentrations

Timmy Neece, Jr*, Melissa Kennedy, Alka Lohmann; Department of Forensic Science, Richmond, VA

Background/Introduction: The International Organization for Legal Metrology (OIML) has established recommendations for breath alcohol testing related to the interference of chemicals that may occur in human breath at the time of sampling. In order for a volatile organic compound to be classified as an interferent it must be found in measurable amounts on the breath of a living person after a 15-20 minute deprivation/observation period and produce a result that is indistinguishable from ethanol. Acetone, methanol, and isopropanol are currently listed as possible interferents and were tested on the Intox EC/IR II by the Virginia Department of Forensic Science. Acetaldehyde was also examined by the laboratory in this study even though it is not currently listed by OIML.

Objective: To evaluate the effects of OIML recommended interfering substances acetone, isopropanol, and methanol on the Intox EC/IR II to ensure accurate and precise testing of evidential breath samples in the Commonwealth of Virginia. Acetaldehyde was also tested, despite its absence from the current OIML interfering compound list.

Method: 0.100 g/210L ethanol stock solution was prepared. The compounds (acetone, isopropanol, methanol and acetaldehyde) were prepared in this ethanol stock and in water (absent of alcohol). The compounds were prepared at the recommended concentrations listed by the OIML and at concentrations below and above the listed concentration. The recommended concentrations are 0.100g/210L for acetone, 0.020g/210L for methanol, and 0.020g/210L for isopropanol. Acetaldehyde is not currently listed as an interferent by OIML but was included in this testing as it was tested by DFS in a previous study. Ten samples were delivered through a Guth 34C simulator into the Intox EC/IR II with Virginia Firmware. In order to meet the recommendations of the OIML, the results of the test with an interfering substance present shall not reflect greater than a 0.021 g/210L variation from the target ethanol vapor value. The acetone (0.050, 0.100, and 0.150 g/210L), methanol (0.010, 0.020, and 0.040 g/210L), isopropanol (0.010, 0.020, and 0.040 g/210L), and acetaldehyde (0.015, 0.032, and 0.075 g/210L) solutions were introduced into five separate breath test instruments.

Result: The testing in Virginia met the recommendations established by OIML for acetone, methanol, and isopropanol because they did not report a sample that exceeded greater than a 0.021 g/210L variation from the target ethanol vapor value. At levels possible in living, conscious humans, the Intox EC/IR II showed no average effect on the reported BrAC or reported the instrument message “Interferent Detected”. During the testing of water mixed with methanol (0.020 and 0.040 g/210L) the instrument reported “Fuel Cell Timeout” during which no numerical result was given due to the fuel cell reaction time taking too long. Acetaldehyde, a compound not listed by the OIML as an interfering substance, reported an elevation of 0.020g/210L at the highest level tested (0.075g/210L in ethanol stock), but this is not considered significant since this and all of the acetaldehyde concentrations tested were higher than those reported in conscious subjects.

Conclusion/Discussion: The Intox EC/IR II with Virginia firmware meets the recommendations by the OIML that the results of the breath test in the presence of interfering substance in a living, conscious person shall not reflect greater than a 0.021 g/210L influence at the OIML specified concentration. This further exemplifies the accuracy and precision of the evidential breath alcohol testing instrument used in the Commonwealth of Virginia.

Keywords: Breath Alcohol, Interfering Substance, Intox EC/IR II

P92
Postmortem Investigation of Newer Antiepileptic Drugs in North Carolina

Sandra C. Bishop-Freeman, **Nina Kornegay*,** Ruth E. Winecker; North Carolina Office of the Chief Medical Examiner, Raleigh, NC

Background/Introduction: Limited postmortem data is available for the newer antiepileptic drugs. These drugs are often used as adjunctive therapy along with other seizure medications. Since 2011, the North Carolina Office of the Chief Medical Examiner has recorded quantitative values for several cases involving zonisamide (n=14), lacosamide (n=14), and oxcarbazepine (n=24). Therapeutic ranges found in the literature along with postmortem values will be evaluated. Typically, with supportive care, overdoses are not fatal however supratherapeutic concentrations of lacosamide and 10-hydroxycarbazepine have been found in recent casework.

Objective: Quantitative data along with informative case studies including a suicidal overdose of lacosamide, will provide the toxicology community with a better understanding of the concentrations of newer antiepileptic drugs found in postmortem cases.

Method: The current procedure in our laboratory uses reference calibrators containing 14 acidic and neutral compounds, however, the procedure utilizes Gas Chromatography-Mass Spectrometry (GC-MS), which has the ability to detect additional compounds. The internal standard mephobarbital is spiked into all vials followed by solid-supported liquid extraction and analysis via gas chromatography with flame ionization detection and GC-MS.

Result:

Drug	Published ~Therapeutic Values (mg/L)	N	Mean Blood (mg/L, mg/kg)	Median Blood (mg/L, mg/kg)	Range (mg/L, mg/kg)
Zonisamide	1.0-30	14	11.1	8.95	4.0-28
Lacosamide	2.5-15	14	15.3	6.50	<5.0-120
10-Hydroxycarbazepine	3.7-37	24	22.1	18.5	<5.0-71

Conclusion/Discussion: The quantitation and interpretation of postmortem antiepileptic drugs may become increasingly important due to the off-label use in mood stabilization, chronic pain and migraines. As a result, therapeutic and supratherapeutic values of lacosamide, zonisamide, and oxcarbazepine metabolite were tabulated.

Keywords: Lacosamide, Zonisamide, Oxcarbazepine

P93
A Case of Death by Flubromazepam and Fluoromethamphetamine

Kimberly Peterson*, James Sailors, Teresa Gray, Alex John; Harris County Institute of Forensic Sciences, Houston, TX

Background/Introduction: There has been an influx in the use of designer amphetamines and benzodiazepines in the United States. Many of these compounds are exclusively manufactured for illicit use. Specifically, flubromazepam and fluoromethamphetamine have no current medicinal use and are produced solely for recreational use. Data demonstrating flubromazepam or fluoromethamphetamine toxicity are scant.

Objective: This case illustrates a fatality in which documented ingestion of fluoromethamphetamine and flubromazepam was confirmed by GC/MS. The decedent, a 53 year old male, was found unresponsive in bed with multiple prescription and illicit drugs in his room. A notepad which listed the time, dosage, and identities of the drugs taken for the month leading up to the decedent's death was found bedside. The following drugs were listed for the day before the decedent was discovered unresponsive: Brintellix, clonidine, etizolam, flubromazepam, 4-fluoromethamphetamine, ethylphenidate, tianeptine, 4-fluorobutyrfentanyl, 3-methoxy phencyclidine, 4-HO-MiPT, and isopropylphenidate.

Method: Blood, vitreous humor, urine, bile, stomach contents, brain, and liver were submitted for toxicological analysis. Immunoassay drug screening was performed on the heart blood using ELISA. Acidic and basic screens were performed on heart blood and stomach contents using GC/MS. A LC-TOF screen for synthetic cannabinoids, designer amphetamines and "z-drugs" was performed on heart blood.

Result: ELISA was presumptively positive for benzodiazepines, amphetamine and methamphetamine in heart blood. Lorazepam was the only benzodiazepine detected by LC/MS/MS, with a reported result of less than 0.010 mg/L in femoral blood. No lorazepam was found in stomach contents at a 1/25 dilution. Lorazepam was not part of the decedent's list, but is a known metabolite of diclazepam. Compounds with similar molecular weights to diclazepam and its metabolites, delorazepam and lorazepam, were identified by LC-TOF. The traditional sympathomimetic amine method for methamphetamine, amphetamine, ephedrine, pseudoephedrine, MDMA, MDA, and phentermine quantitation was negative in blood and stomach contents. An LC-TOF screen was also negative; however, designer benzodiazepines and many novel psychoactive substances are not reportable from the LC-TOF screen. The TOF screen was searched using the molecular weights of vortioxetine (Brintellix), etizolam, 4-fluoromethamphetamine, ethylphenidate, tianeptine, 4-fluorobutyrfentanyl, 3-methoxy phencyclidine and isopropylphenidate. Peaks corresponding to those molecular weights were identified but not reported as there was no confirmatory test available that would corroborate initial findings. This lack of ability to report from LC-TOF does not extend to the basic drug screen. The basic drug screen detected both flubromazepam and fluoromethamphetamine in the heart blood and stomach contents.

Conclusion/Discussion: The cause of death was determined to be, "Acute combined toxic effects of flubromazepam and fluoromethamphetamine." The manner of death was ruled accidental.

Keywords: Flubromazepam, Fluoromethamphetamine, Designer Drugs

P94

Immunoassay for the Detection of Hydrocodone and Hydromorphone in Urine on Automated Clinical Analyzers

Steven Vu, Chi-Yun Pai, Amy Lu, **Bernice Lin***, Marie Lin; Lin-Zhi International, Inc., CA

Background/Introduction: The LZI Hydrocodone assay is a homogeneous enzyme immunoassay for the semi-quantitative and qualitative detection of hydrocodone and hydromorphone in urine on clinical analyzers. The assay is based on competition between hydrocodone and hydromorphone in the sample and hydrocodone-labeled glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody in the reagent. Two assays are available, at 100 ng/mL cutoff (assay range 5-300 ng/mL) and at 300 ng/mL cutoff (assay range 10-800 ng/mL).

Method/Result: For 100 ng/mL Cutoff: Based on semi-quantitative mode (five-point calibration) with hydrocodone on Beckman Coulter AU480® (n=88), control samples concentrations at the cutoff and ±25 % of the cutoff showed mean recoveries of 75 ng/mL (99.5%), 100 ng/mL (100%), and 125 ng/mL (100.2%). Within-run precision (n=22) showed %CV values of 1.7%, 1.5%, and 1.2%, respectively. Between-run precision (n=88) showed %CV values of 2.4%, 1.9%, and 1.9%, respectively. Assay specificity showed cross-reactivities of hydromorphone (85.1%), hydromorphone glucuronide (52.2%), dihydrocodeine (5.0%), norhydrocodone (0.6%), codeine (3.5%), codeine-6-glucuronide (0.6%), morphine (2.0%), morphine-3-glucuronide (0.8%), oxycodone (2.1%), oxymorphone (1.3%).
A set of 80 clinical samples (40 positive and 40 negative samples) were tested in a Method Comparison study. Clinical samples of hydrocodone or hydromorphone were tested and confirmed by GC or LC/MS: 20 drug free samples 4 samples between 0.1 – 49.9 ng/mL; 16 samples between 50 to 99.9 ng/mL (3 false positives); 8 samples between 100 to 149.9 ng/mL (3 false negatives); 32 samples greater than 150 ng/mL. Clinical sample study showed a 92.5% agreement for both positive and negative samples as compared when run in both qualitative and semi-quantitative modes.
For 300 ng/mL Cutoff: Based on semi-quantitative mode (five-point calibration) with hydrocodone calibrators on Beckman Coulter AU480® (n=88), control samples at the cutoff and ±25 % of the cutoff showed mean recoveries of 225 ng/mL (102.2%), 300 ng/mL (100.3%), and 375 ng/mL (103.6%). Within-run precision (n=22) showed %CV values of 1.2%, 1.3%, and 1.2%, respectively. Between-run precision (n=88) showed %CV values of 1.5%, 1.9%, and 1.7%, respectively. Assay specificity testing showed cross-reactivities of hydromorphone (78.1%), hydromorphone glucuronide (49.2%), dihydrocodeine (3.3%), norhydrocodone (0.5%), codeine (2.2%), codeine-6-glucuronide (0.4%), morphine (1.3%), morphine-3-glucuronide (0.7%), oxycodone (1.4%), oxymorphone (0.9%).

A set of 80 clinical samples with the following concentrations of hydrocodone or hydromorphone were tested and concentrations also confirmed by GC or LC/MS. 20 drug free samples; 12 samples 0.1 – 149.9 ng/mL; 8 samples 150 to 299.9 ng/mL (2 false positives); 8 samples between 300 to 449.9 ng/mL (2 false negatives); 32 samples more than 450 ng/mL. Clinical sample study showed a 95.0% agreement for both positive and negative samples as compared when run in both qualitative and semi-quantitative modes.

Conclusion/Discussion: For Both Cutoffs: no interference from a panel of 45 structurally unrelated drug compounds, sample pH value (range of 3 to 11), sample specific gravity (1.000 to 1.030), and endogeneous compounds tested. In conclusion, the assays are shown to produce accurate and reliable results and are very sensitive assays for hydrocodone and its metabolites with low interference from other opiates.

Keywords: Immunoassay, Hydrocodone, Hydromorphone

P95
Creating and Utilizing HRAM Orbitrap MS/MS and MSn Libraries for Unknown Analysis

Marta Kozak, Tim Stratton, **Kristine Van Natta***; Thermo Fisher Scientific, San Jose, CA

Background/Introduction: Targeted screening for known compounds of interest only covers a portion of the need for the analysis of complex samples. Untargeted techniques which look for potential unknowns of interest is a key aspect which attempts to address new emerging compounds (designer drugs or doping agents) or metabolites / degradants of known compounds of interest.

Objective: To demonstrate the application of a diversity fragmentation library to identify compounds from multiple chemical classes in a biological matrix and to discuss the creation of such a library.

Method: To construct the library, fragmentation data was acquired for several thousand compounds which were grouped into sixteen major chemical classes based on their application including such groups as therapeutic drugs, drugs of abuse, endogenous compounds, personal care products, etc. This data covered an extended fragmentation energy range and two different activation mechanisms and was subsequently curated for noise removal and spectral recalibration. The recalibrated data was hosted on a cloud based service with an application program interface which allowed the fragmentation library to be searched through a desktop application. This library is under continuous growth, but at the time of data analysis for this work contained approximately 6,000 compounds. Multiple samples of human urine were prepared for untargeted analysis by HRAM LC-MS. The samples were prepared by centrifugation to remove particulates followed by dilution. Separation was performed by UHPLC with a linear gradient over a period of 10 minutes and an overall 15 minute run time (initial conditions of 95A:5B to maximum conditions of 5A:95B, A - water with 0.1% formic acid, B - acetonitrile with 0.1% formic acid). Data was collected with a full scan data with resolution of 70K (FWHM at 200m/z) and data dependent MS/MS spectra with resolution of 35K. Fragmentation spectra of detected peaks were searched against the library using an identity approach where the precursor molecular weight and fragmentation spectra were required to match. Subsequent to this, a similarity search for the remaining compounds was also compared where the fragmentation data for the unknown had to be similar to potential structurally related library results.

Result: Depending on the sample analyzed, between 2,000 to 3,500 unique molecular weights were identified. Across the samples, identities could be proposed for between 300-400 compounds in the urine samples by the identity search, with an additional 250-500 compounds having tentative information from the similarity search. The majority of compounds identified were endogenous metabolites while plasticizers, food additives, and personal care product ingredients were the next three major chemical classes. In total, an average of 20-30% of all unknown compounds in the urine samples could have a tentative identity assigned to them based on either identity or similarity to the HRAM fragmentation library.

Conclusion/Discussion: Unknown analysis of complex samples presents a wide range of challenges. Often, most of the compounds present in the sample are of no interest in the context of the analysis at hand, being compounds expected to be in the sample or compounds that are not of interest. Determining the “unknowns of interest” can be difficult, and the tools discussed here attempt to enable this by providing a means to identify what may be compounds that are of no interest, thus reducing the total number of unknown compounds with the samples that will require further investigation.

Keywords: Mass Spectrometry, Unknown Analysis

P96

Qualitative Identification of Heroin and Synthetic Opiates in Seized Drug Samples Using Ambient Ionization High Resolution Time-of-Flight Mass Spectrometry

Jamie Foss*[1], Amanda Moore[2], Sabra Botch-Jones[2], Frank Kero[3]; [1]PerkinElmer, Shelton, CT, [2]Boston University School of Medicine, Boston, MA, [3]PerkinElmer, Downers Grove, IL

Background/Introduction: The northeastern United States has seen a major increase in abuse and number of overdoses from heroin and fentanyl in the past three years. With this increase has also come an increase in new synthetic opiates and fentanyl analogues which have been the cause of many drug overdoses. Current methodologies include targeted analysis using immunoassay, GC-MS, and LC-MS/MS. These techniques may not allow for identification of unknowns, and may lead to analytes of interest not being detected throughout the course of employing a traditional analytical scheme. An alternative to these techniques is Direct Sample Analysis (DSA) Time-of-Flight Mass Spectrometry (TOF), which uses a modified atmospheric pressure chemical ionization source coupled to a time-of-flight mass spectrometer. DSA allows for the identification of knowns and unknowns using small sample volumes, minimal sample preparation, and short analysis times to generate accurate mass data for the identification of both knowns and unknowns. The data generated is completely reviewable, allowing for retrograde analysis of compounds without the need for re-analysis of the sample. Samples can be rapidly screened and later confirmed by comparing reproducible fragmentation patterns from in-source Collisionally Induced Dissociation (CID) to known analytical reference materials.

Objective: Explore the suitability of DSA-TOF for the qualitative identification of heroin and synthetic opiates in forensic case samples. Heroin, fentanyl, and acetyl fentanyl were assessed. Selectivity, reproducibility, matrix effects, limits of detection, and robustness were evaluated as part of a method validation study.

Method: The instrument used was a PerkinElmer AxION 2 TOF MS coupled to a PerkinElmer AxION DSA module. Instrument conditions were as follows: a corona current of 5 µA, heater temperature of 325°C, auxiliary gas (N_2) pressure of 80 psi, drying gas (N_2) flow of 3 L/min, and drying gas (N_2) temperature of 25°C. The AxION 2 TOF MS was run in positive mode with a flight tube voltage of -10,000 V. The capillary exit voltage was set to 170 V for MS analysis. Mass spectra were acquired with a mass range of 70-2000 *m/z* and an acquisition rate of 10 spectra/sec. To maintain mass accuracy, two lock mass ions were used (*m/z* 121.0509 and *m/z* 322.0481). All samples were analyzed for only 20 seconds. Sample preparation was simple: a small amount of powder was added to 3 mL of LC-MS grade water. 5 □L of the solution was spotted directly on to the mesh target screen.

Result: Heroin, fentanyl, and acetyl fentanyl parent and fragment ions were detected with mass accuracies below 2 mDa, with the parent ion often below 5 ppm. Limits of detection for each analyte ranged from 1 ppm to 10 ppm. In a parallel casework study, DSA-TOF showed good agreement to forensic casework samples where heroin, fentanyl, and acetyl fentanyl were found. In several cases, the DSA-TOF identified fentanyl and/or acetyl fentanyl mixed with heroin, where only heroin was previously identified and reported. DSA also provided additional sample information through the detection of adulterants that were not previously detected using the traditional analytical scheme.

Conclusion/Discussion: DSA was found to be suitable for the rapid screening of heroin and synthetic opiates in seized drug samples. Further confirmation using in-source CID can be accomplished by increasing the capillary exit voltage from 80V to 170V and comparing to analytical reference materials. Fentanyl and acetyl fentanyl were found to have the same soft fragmentation ions from in-source CID; however, these compounds could be distinguished by exact mass of the parent compound.

Keywords: DSA-TOF, Synthetic Opiates, Drug Analysis

P97

Quantification of Eight Synthetic Piperazines in Biological Specimens Using UFLC-ESI-MS/MS

Raquel LeBlanc, **Sabra Botch-Jones***; Boston University School of Medicine, Boston, MA

Background/Introduction: Synthetic piperazines are chemically-produced compounds that contain a six-member ring with two opposing nitrogen atoms. Several piperazine derivatives, namely 1-benzylpiperazine (BZP), 1-(3-trifluoromethylphenyl)-piperazine (TFMPP), and 1-(3-chlorophenyl)-piperazine (*m*CPP), have fallen into the "designer drugs" category due to their increasing recreational use as a "legal" alternative to ecstasy (3,4-Methylenedioxymethamphetamine). These compounds share similar physiological effects with amphetamines which make them desirable to young adults in party-type atmospheres. BZP, a Schedule I drug for its high abuse potential and no accepted medical use, is the only recreationally-abused synthetic piperazine federally controlled in the United States.

Objective: The purpose of this research was to develop and validate a reliable method to identify and quantify eight forensically significant synthetic piperazines in blood and urine using ultra-fast liquid chromatography-electrospray ionization-tandem mass spectrometry (UFLC-ESI-MS/MS). The method was validated according to SWGTOX guidelines for quantitative analysis for both matrices and includes the following analytes: 1-benzylpiperazine (BZP), 1-(4-fluorobenzyl)-piperazine (FBZP), 1-(4-methylbenzyl)-piperazine (MBZP), 1-(4-methoxyphenyl)-piperazine (MeOPP), 1-(*para*-fluorophenyl)-piperazine (*p*FPP), 1-(3-chlorophenyl)-piperazine (*m*CPP), 2,3-dichlorophenylpiperazine (DCPP), and 1-(m-trifluoromethylphenyl)-piperazine (TFMPP).

Method: All samples were prepared by fortifying 100µL of certified drug-free whole blood and urine (UTAK, Valencia, CA, U.S.A.) with certified reference standards (Cayman Chemical, Ann Arbor, MI, U.S.A.) of each analyte at desired concentrations and standard additions of benzylpiperazine-D7, 1-(3-chlorophenyl)-piperazine-D8, and 3-trifluoromethylphenylpiperazine-d4 internal standards (Cerilliant, Round Rock, TX, U.S.A). After pretreatment with 1mL phosphate buffer, samples underwent solid phase extraction (SPE) on mixed-mode copolymeric columns (Clean Screen®, UCT Inc., Levittown, PA, U.S.A.). Eluents were evaporated to dryness with low heat (65°C) and reconstituted with a 50:50 mixture of methanol and 2mM ammonium formate buffer with 0.2% formic acid before being analyzed by a UFLC (Shimadzu Corporation, Kyoto, Japan) with 4000 QTRAP ESI-MS/MS (SCIEX™, Framingham, MA, U.S.A.) system. Analyses were performed with multiple reaction monitoring scans in positive ionization mode using ions and voltages obtained from a manual compound optimization. Analytes were separated on a reversed-phase column (Kinetex® F5, Phenomenex®, Torrance, CA, U.S.A.) with a binary gradient consisting of a 2mM ammonium formate buffer with 0.2% formic acid and methanol with 0.1% formic acid. The flow rate was 0.400 mL/minute. Analyst™ (SCIEX) software was used for data collection and MultiQuant™ (SCIEX) software was used for quantitation.

Result: The total run time was 11.5 minutes. All calibration curves exhibited R^2 values > 0.99 using a 1/x weighting factor. A linear dynamic range of 20-2000 ng/mL was used for all analytes in both matrices, except for BZP in urine which ranged from 50-2000 ng/mL. In blood, the limit of quantitation was 10 ng/mL for *m*CPP and TFMPP and 20 ng/mL for BZP, FBZP, MBZP, MeOPP, *p*FPP and DCPP. In urine, the limit of quantitation was 10 ng/mL for MeOPP, *m*CPP, TFMPP and DCPP, 20 ng/mL for FBZP, MBZP and *p*FPP and 50 ng/mL for BZP. When evaluating a 200 ng/mL concentration, the SPE procedure showed percent recoveries of 80-95% for blood; except for BZP, FBZP, and MeOPP which had recoveries of 60%, 60%, and 105%, respectively. Percent recoveries ranged from 82-94% for urine; except for BZP and FBZP which had recoveries of 66% and 68%, respectively. Bias and precision were assessed at concentrations of 50, 200, and 700 ng/mL. All QC samples were calculated within ±20% bias and ±20% coefficient of variation. The highest concentration evaluated that did not produce carryover in subsequent matrix blanks was 5000 ng/mL. Ionization was suppressed for all analytes in both matrices by 45-95%.

Conclusion/Discussion: This is a sensitive, reliable, and robust method with a wide linear dynamic range to account for the presence of these analytes in both blood and urine. This research will provide for the confirmatory identification of these substances in forensic casework.

Keywords: Designer Drugs, Synthetic Piperazines

P98

Re-evaluation of Conditions for Hydrolysis of Glucuronides Prior to LC-MS/MS Screening of Urine Samples for Drugs of Abuse

Mumtaz Pasha*, Kathryn Fuller; Minnesota Bureau of Criminal Apprehension, St. Paul, MN

Background/Introduction: The toxicology section of the Minnesota Bureau of Criminal Apprehension Forensic Science Laboratory switched from immunoassay to an LC-MS/MS based method to screen urine samples for basic drugs of abuse in 2011. Following hydrolysis with β-glucuronidase and dilution, urine samples are analyzed on a Shimadzu Prominence HPLC coupled to a Sciex QTRAP 4500 System. An Ultra Biphenyl Column (50 x 2.1 mm, 5 µm) (Restek) is used with a scouting gradient to separate the >90 analytes that are targeted by the screening procedure. To mitigate HLPC column fouling caused by the enzyme, acetonitrile is added and the samples are centrifuged to pellet the precipitated protein. Utilizing this procedure, approximately 100 case samples can be tested before the performance of the column becomes unacceptable.

Objective: In an attempt to extend column life without adopting additional sample preparation steps, the sample hydrolysis procedure was re-evaluated to determine whether less enzyme could be used without compromising the ability to detect highly conjugated analytes.

Method: Hydrolysis was assessed using two pools of urine that were supplemented with drug standards. The first pool was supplemented with glucuronides such that the total concentration of glucuronides in the urine was 4.21 mg/L. The glucuronides that were included were chosen based on frequency of detection and commercial availability. The glucuronides used were: 11-nor-Δ9-THC-9-carboxylic acid glucuronide, hydromorphone-3-β-D-glucuronide, morphine-3-β-D-glucuronide, oxymorphone-3-β-D-glucuronide, lorazepam glucuronide, oxazepam glucuronide and temazepam glucuronide. The second pool was supplemented with the corresponding free drugs that were added at equimolar concentrations to the glucuronide standards in the first pool (11-nor-Δ9-THC-9-carboxylic acid, hydromorphone, morphine, oxymorphone, lorazepam, oxazepam and temazepam). Samples from the two pools of urine were subjected to identical hydrolysis procedures and analyzed by LC-MS/MS. MRM scans were used to detect the free drugs. After normalization to internal standards, the chromatographic peak areas of the free drugs from the two pools of urine were compared to measure hydrolysis yield using the following equation: hydrolysis yield = peak area of the free drug in urine supplemented with glucuronides ÷ peak area of the free drug in urine supplemented with the free drugs. A hydrolysis yield of 100% is interpreted as complete hydrolysis of a glucuronide bound drug. Three β-glucuronidase sources were evaluated: *Haliotis rufescens*, *Helix pomatia* and *Patella vulgata* and enzyme activity concentrations and hydrolysis times were varied. The hydrolysis conditions that are currently in use are: 17 kU/mL β-glucuronidase from *H. pomatia* at pH 5.0 incubated at ~62 °C for 45 minutes. These conditions were more than adequate to effect near 100% hydrolysis of the glucuronide standards. Extension of the hydrolysis period to 60 minutes allowed the enzyme concentration to be decreased to 3.3 kU/mL with hydrolysis still proceeding to near completion. β-glucuronidase from the three sources performed equivalently under the conditions that were studied. Samples collected from human subjects were then tested to determine whether the conditions that were optimal for hydrolysis of spiked samples were also adequate for samples that more closely approximate case samples.

Result: The human subjects' samples introduced matrix diversity and included a greater variety of analytes at a range of concentrations. Varied enzyme activity concentrations and hydrolysis times were evaluated for the three enzyme sources by comparison of the normalized MRM chromatographic peak areas of the free drugs in the samples. Based on these analyses, it was determined that treatment with 6.7 kU/mL β-glucuronidase from any of the three sources at ~62 °C for 60 minutes was as effective as the hydrolysis conditions that are currently used.

Conclusion/Discussion: Adopting these hydrolysis conditions will reduce the cost per sample analyzed. Furthermore, the ability to use enzyme from multiple sources will introduce purchasing flexibility and will buffer the laboratory from enzyme supply chain issues. The revised procedure has not been in use long enough to determine conclusively whether it will extend the HPLC column life, this is still being evaluated.

Keywords: Hydrolysis, Urine, LC-MS/MS

P99
Differential Cross-Reactivity of THC-COOH Enantiomers in Cannabinoids EIA

C. Clinton Frazee III*, Robert Krumsick, Uttam Garg; Department of Pathology and Laboratory Medicine, Children's Mercy Hospitals and Clinics, Kansas City, MO

Background/Introduction: The National Institute of Justice lists marijuana as the most commonly used illicit drug and its usage has been increasing among adolescents and young adults. Marijuana is legalized for recreational use in Colorado, Washington, Oregon and Alaska. In addition, 24 states and the District of Columbia have enacted laws which legalize medical marijuana. Enzyme immunoassays (EIA) remain one of the most common methods for detecting marijuana use. Cannabinoids EIA are responsive to the 11-nor-9-Carboxy- delta9-THC (THC-COOH) metabolite, but they are not necessarily equally responsive to all enantiomers, glucuronides and enantiomer combinations of THC-COOH.

Objective: This study compared the specificity of Siemen's Cannabinoids EIA for d-THC-COOH-glucuronide, l-THC-COOH, and d,l-THC-COOH.

Method: Testing was performed using a Siemen's (formerly Dade Behring) dual channel V-Twin Drug Testing System and Siemen's cannabinoids assay calibrated with a 20 ng/mL cutoff for THC-COOH. The package insert does not identify the THC-COOH enantiomer for this assay. Cerilliant standards for d-THC-COOH-glucuronide, l-THC-COOH and d,l-THCOOH were used to spike negative UTAK urine at concentrations ranging from 10-1000 ng/mL.

Result/Discussion: It is widely known that the d-enantiomer form of most drugs is typically the more active form. Fortunately, the availability of drug enantiomers standards and reference material is much better today than it was even a decade ago. Our laboratory analyzes urine, serum, blood and meconium for drugs of abuse by EIA. Non-urine matrices require spiking in-house calibrators and controls in the appropriate matrix for testing. We have noticed that false-positive cannabinoids in the non-urine matrices occurred much more frequently than with urine alone. As a result of this study, we determined that the l-THC-COOH spiked urine yielded change in absorbance levels which were more consistent with our expectations at the various spiked concentrations. It was determined that l-THC-COOH is more cross-reactive than d,l-THC-COOH which is more cross reactive than d-THC-COOH-glucuronide. Literature supports this finding insomuch as l-THC has been shown to exhibit a higher affinity for the body's cannabinoids receptors. Upon switching to l-THC-COOH standards for blood calibrator preparation, we noticed a significant decrease in false positive cannabinoids EIA results with our postmortem cases.

Conclusion: Toxicology laboratories receive a variety of matrices for testing and using whole blood for EIA drugs of abuse testing is a common practice. When preparing in-house calibrators and evaluating EIA specificity for a drug, it is important to select the appropriate enantiomer for which the assay was designed. We determined that the l-THC-COOH enantiomer provides the most specific response when using the Siemen's Cannabinoids Assay.

Keywords: EIA, THC-COOH, Enantiomer

P100
Detection of Vecuronium and Its Metabolite in Two Acute Intoxication Cases

Sungmin Moon*, Ilwoong Seol, Yuran Park, Hyesun Yum, Minji Kang, Meejung Park, Sangki Lee; Forensic Toxicology & Chemistry Division, Seoul Institute, National Forensic Service, Seoul, Korea

Background/Introduction: Vecuronium is one of the muscle relaxants which block the effect of acetylcholine. Vecuronium should be always applied with mechanical ventilation because even if the muscle relaxant is administered in a recommended dosage, patients can have difficulty breathing. Vecuronium has been used in suicide attempts by medical professionals such as doctors and nurses.

Objective: Because vecuronium is rapidly degraded to 3-desacetylvecuronium, a rapid and accurate method for simultaneous determination of vecuronium and its metabolite is essential. This report presents two suicidal cases involving a nurse and a doctor who died of self-administration of vecuronium.

Method: The concentration of vecuronium and 3-desacetylvecuronium in each blood sample was measured by liquid chromatography-tandem mass spectrometry (LC/MS/MS) after deproteinization with acetonitrile. Mivacurium was used as an internal standard. Vecuronium and 3-desacetylvecuronium were separated using a Syncronis Hilic column (2.1*100 mm, 1.7 um) with gradient mobile phase elution. Vecuronium and its metabolite were detected in ESI positive mode. Molecular ions (m/z 558.2, 473.1 and 221.6 for vecuronium, metabolite and the IS respectively), were selected in Q1 and the corresponding daughter ions (m/z 99.9 and 357.2 for vecuronium and m/z 100.2 and 356.2 for metabolite and m/z 179.3 for the IS) were detected in Q3. This method was validated for linearity, precision, accuracy for each compound.

Result: Good linearity was obtained with correlation co-efficient (R^2) of 0.995 for vecuronium and metabolite and the method was validated by evaluating the selectivity, precision and accuracy. Accuracy at three different concentrations was 88.1-98.1% and 87.3-112.0%, the precision was 1.9-6.1% and 4.0-6.1% for vecuronium and metabolite respectively.

Conclusion/Discussion: In these cases, vecuronium and its metabolite were identified and quantitated through analysis of postmortem specimens; heart blood, peripheral blood, gastric contents, aqueous humor, liver and kidney. The concentrations of vecuronium in heart blood and peripheral blood were 0.13 mg/L and 0.056 mg/L in Case 1, and the concentrations of vecuronium in heart blood, liver and kidney were 0.30 mg/L, 0.64 mg/kg and 0.20 mg/kg, respectively in Case 2. 3-Desacetylvecuronium was also detected in the postmortem specimens in both Case 1 and Case 2. This method can be successfully used to detect vecuronium and its metabolite in biological samples.

Keywords: Vecuronium, Intoxication, LC/MS/MS

P101
The Case of Pesticide Poisoning from a Soft Drink Containing Methomyl

Sanghee Woo*, Joon-Bae Lee, Se Yeon Park, Dong-Eun Park, Kyung-Suk Oh, Wonkyung Yang; Daegu Institute, National Forensic Service, Chilgok, Korea

Background/Introduction: Methomyl is one of the highly toxic carbamate insecticides first registered in 1968. It is a broad spectrum insecticide used in a wide variety of treatment applications. Methomyl is a highly toxic inhibitor of cholinesterase, an essential nervous system enzyme. Methanol is the common ingredient in a liquid formulation of methomyl. It was reported that the six elderly women lost consciousness while foaming at the mouth after sharing a soft drink at a community center in a southeastern city of Korea. Two died and four others fell into critical condition. Methomyl was detected in the soft drink bottle which is speculated that they drank.

Objective: The objective was to determine methomyl and methanol in the blood of the victims.

Method: Methomyl in the blood samples was measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS) after deproteination with a methanol:acetonitrile (1:1) mixture. The LC system was coupled to a 4500 QTrap mass spectrometer using electrospray ionization in positive mode and multiple reaction monitoring (MRM) mode. Liquid-chromatographic analysis was performed in gradient mode on a Kinetex C18 column (2.6μm, 50 x 2 mm). The mobile phase consisted of 10 mM ammonium formate in water and a methanol : acetonitrile (1:1). For analysis of methanol, it was used headspace-gas chromatography (HS-GC). The column was a Carbowax 20M (2m x 1/8”). The chromatographic conditions were as follows: detector, FID at 240°C; injector temperature, 240°C; oven temperature, 100°C isothermal; carrier gas, helium at a flow of 25mL/min.

Result: The concentrations of methomyl are shown in the table below.

	Victim 1	Victim 2	Victim 3	Victim 4	Victim 5	Victim 6
Methomyl Concentration (μg/mL)	0.04	0.50	1.18	1.90	5.38	9.53

Victim 1 and the oldest victim 4 died, and the others recovered after receiving treatment. It was reported that blood methomyl concentrations in 10 adults who died after the accidental or intentional ingestion of an overdose average 26 mg/L (range 8.0~57 mg/L). Methanol was not detected from bloods of all victims. It was inferred that the product used in the crime was a powdered formulation or evaporated methanol by long time careless storage.

Conclusion/Discussion: It was suggested that victim’s physical condition has an effect on recovery or death besides the blood concentration of methomyl. Caution must be taken by laboratories when it evaluates the toxicity of chemical substances detected in the bloods.

Keywords: Methomyl, Methanol, LC-MS/MS

P102

A Case Study on the Fire Victim in the Vehicle by GC/MS through Derivatization of Cyanide with Pentafluorobenzyl Bromide (PFBBr)

Joon-Bae Lee[1], **Sanghee Woo[1]*,** Se Yeon Park[1], O-Seong Kwon[1], Ki Jung Paeng[2]; [1]Daegu Institute, National Forensic Service, Chilgok, Korea, [2]Department of Chemistry, Yonsei University, Wonju, Korea.

Background/Introduction: Hydrogen cyanide (HCN) is an extremely toxic gas and it could be frequently produced from the incineration of plastics such as ABS (acrylonitrile-butadiene-styrene) and subsequently after the fire victim inhaled the smoke, cyanide could be found in the blood. Therefore, in addition to the carboxyhemoglobine (COHb) test in blood samples, HCN could be used as a good marker for the postmortem examination in fire death. In the fire case, a charred body was found inside a burned vehicle with a suicide note. Even though COHb value is one of the conclusive evidences, measuring COHb in denatured blood might be difficult due to the formation of methemoglobin (MetHb) or severely thermal denaturation of blood proteins. To overcome this difficulty, cyanide could be used as an indicator for investigating fire victim case.

Objective: The objective in this study was to determine cyanide in blood of a fire victim.

Method: In this study, gas chromatography/mass spectrometry (GC/MS) was adopted to measure cyanide in the blood through derivatization with pentafluorobenzyl bromide (PFBBr) under cation surfactant by scan and SIM mode. The internal standard used was tribromobenzene (TBB). The chromatographic conditions were as follows: injector temperature, 250°C; initial temperature, 80°C; ramp, 10°C/min; final temperature, 280°C for 3min; carrier gas, helium at a flow of 1.0mL/min; split ration, 1:10.

Result: The cyanide detection limit was 0.01 μg/mL using SIM mode in the GC/MS analysis. The concentration of cyanide in the heart blood and cerebral blood of the victim was found to be 0.36 μg/mL and 1.20 μg/mL, respectively. It was reported that whole blood cyanide in 10 nonsmokers was found to average 0.016 μg/mL, whereas in 14 smokers the mean level was 0.041 μg/mL.

Conclusion/Discussion: It was suggested that cyanide can be substituted for COHb in the blood of victims who died in a fire.

Keywords: Fire Victim, Cyanide, Pentafluorobenzyl Bromide (PFBBr)

P103

Playing with Probability, Using Low Probability Isotopes to Extend the Dynamic Range of an LCMS Assay for Amphetamine and Methamphetamine

Anna Miller, Melissa Goggin, An Nguyen, **Gregory C. Janis***; MEDTOX Laboratories, Laboratory Corporation of America® Holdings, St. Paul, MN

Background/Introduction: Toxicology testing is faced with the conundrum of being acutely focused on achieving ultimate analytical sensitivity detecting the lowest possible levels of drugs while at the same time also being tasked with analyzing samples possessing drug levels at concentrations tens of thousands times higher than the LLOQ. The wide breadth of concentration exceeds the linear dynamic range of available instrumentation. In addition, high drug concentration can deteriorate chromatographic peak shape, shift peak retention time, and alter fragmentation ratios. Current strategies for measuring samples possessing analyte concentrations in excess of the upper limit of linearity rely on sample re-extraction and re-analysis, which may significantly delay reporting results.

Objective: We set to develop a procedure of dynamically extending the upper linear regions of LC-MS/MS quantitation without reanalysis. We propose exploiting the natural, low abundance isotopes of targeted analytes as a linear extension above the normal linear quantitation based upon the most common analyte isotope.

Method: For any given naturally occurring carbon atom, there is a 1.109% probability that the nucleus contains 7 neutrons and thus, the specific carbon atom possesses an atomic mass of 13. The probability that an organic compound contains one or two ^{13}C atoms is proportional to the number of carbons in the molecule as each probability event is independent. From a different perspective, in any given population of natural molecules, there are a small percentage of molecules containing one ^{13}C atom and a smaller population containing two ^{13}C atoms. For amphetamine (containing 9 carbons) and methamphetamine (with 10 carbons), 0.44% and 0.54% of the molecules will contain two ^{13}C atoms.
We have developed and validated a LC-MS/MS methodology where we sequentially monitor the predominant (all ^{12}C) isotopes of amphetamine and methamphetamine in urine. In samples where the concentration of the analytes approaches the upper limits of linearity and chromatographic performance the assay switches to monitor the low probability $^{13}C_2$ isotopes. Although the two ranges combine to form one method, the two halves were essentially independently validated.
Solvent clarified urine samples are analyzed by UPLC-MS/MS on a system comprised of an Acquity® UPLC (Waters) and a Sciex 5500® QQQ. Amphetamine and methamphetamine are quantified over a range of 10 to 1000 ng/mL using a transition with precursors of 136.1 and 150.1 m/z , which comprise the predominant ^{12}C isotope. When the signal reaches an intensity approaching 1000 ng/mL the mass spectrometer is triggered to monitor transitions with precursors of 138.1 and 152.1 m/z corresponding to natural but low abundance molecules containing two ^{13}C atoms. The triggered, heavy isotope transitions cover the analytical range of 1,000 to 100,000 ng/mL. With the exception of the isotope mass, all instrumental parameters for the monitored transitions of the two isotopes are identical.

Result: Patient samples with elevated screening results for amphetamine were evaluated using the validated $^{13}C_2$ analysis method and were accurately quantified well above the normal linear range for ^{12}C transitions. Furthermore, two additional $^{13}C_2$ transitions were monitored for qualifying ratios. Use of the low probability isotope not only extends the analytical range of quantitation by 100 fold, it also allows the successful application of qualitative criteria such as retention time, peak shape and MRM ratios to samples with excessively high analyte concentrations.

Conclusion/Discussion: In summary, we have successfully utilized the natural low abundance of heavy isotopes to create a two segment amphetamine and methamphetamine assay capable of successfully identifying and quantifying the analytes over an extended dynamic range of 10 to 100,000 ng/mL. The effective 100 fold increase in the functional dynamic range of the assay reduces the need for sample reanalysis and improves sample turn-around time.

Keywords: Mass Spectrometry, Dynamic Range, Carbon-13

Author Index

Author Index

Author Index

Author Index

Author Index