Sunday, October 18, 2015

- Registration Opens (8:00am-6:00pm)
- NSC-ADID Meeting (8:00am-12:00pm)
- NLCP Inspector Training (2:00pm-6:00pm)
- YFT Symposium (5:00pm-9:00pm)
- Dinner On Your Own
- TBD-Immunalysis hosted Sun. Eve. Reception (6:00pm-9:00pm)

Monday, October 19, 2015

- Continental Breakfast (7:00am-8:30am)
- Registration (7:00am-6:00pm)
- ABFT Exam Committee (8:00am-5:00pm)
- Student Enrichment Progr. (8:00am-5:00pm)
- SOFT Workshops (8:00am-5:30pm)
- ABFT Lab. Accred. Comm. (8:00am-5:00pm)
- Lunch On Your Own
- ABFT Exec. Comm. Meet (12:00pm-5:00pm)
- SOFT-AAFS Drugs & Driving (5:30pm-7:00pm)
- Dinner On Your Own

Tuesday, October 20, 2015

- Continental Breakfast (7:00am-8:30am)
- Registration (7:00am-6:00pm)
- SOFT Board Meeting (7:00am-12:00pm)
- SOFT Workshops (8:00am-5:30pm)
- ABFT Exam (8:00am-12:00pm)
- ABFT Lab. Accred. Comm. (8:00am-12:00pm)
- ABFT Board Meeting (12:00pm-5:00pm)
- Welcome Recep.w/Exhibits (6:30pm-9:00pm)
- Sunshine/Rieders Auction (6:30pm-8:00pm)
- Professional Develop. Fair (6:30pm-8:00pm)
- Elmer Gordon Forum (8:00pm-9:30pm)
- TBD-SOFToberfest Reception, hosted by Thermo Fisher Scientific (9:30pm-mid)

EXHIBITS OPEN

Tuesday – 6:30pm-9:00pm
Wednesday – 7:00am-5:00pm
Thursday – 7:30am-3:30pm

Update – Sept. 17, 2014

Wednesday, October 21, 2015

- Registration (7:00am-5:00pm)
- Exhibit Hall/Auction Open (7:00am-5:00pm)
- Continental Breakfast (7:00am-8:30am)
- JAT/OUP Breakfast Meeting (7:00am-8:00am)
- Opening Ceremony (Plenary) (8:00am-9:00am)
- Scientific Session #1 (9:00am-10:00am)
- Refreshment Break (10:00am-10:30am)
- Scientific Session #2 (10:30am-12:00pm)
- Lunch with Exhibitors (12:00pm-1:30pm)
- Poster Session #1 in Exh. Hall (12:00pm-1:30pm)
- Scientific Session #3 (1:30pm-3:00pm)
- Refreshment Break (3:00pm-3:30pm)
- Scientific Session #4 (3:30pm-5:00pm)
- Explore the Georgia Aquarium (6:00pm-10:00pm)
- TBD-Nite Owl "XV" Reception, hosted by Cerilliant (10:00pm-mid)

Thursday, October 22, 2015

- Registration (7:00am-5:00pm)
- Karla Moore Fun Run/Walk (6:30am-8:00am)
- Continental Breakfast (7:00am-8:30am)
- AAFS Steering Committee (7:00am-9:00am)
- Exhibit Hall Open (7:00am-3:30pm)
- Silent Auction Open (7:00am – 1:30pm)
- Exhibitor Feedback Meeting (8:00am-9:30am)
- SWGTOX update (8:00am-8:30am)
- Scientific Session #5 (8:30am-10:00am)
- Refreshment Break (10:00am-10:30am)
- Scientific Session #6 (10:30am-12:00pm)
- Lunch with Exhibitors (12:00pm-1:30pm)
- Poster Session #2 in Exh. Hall (12:00pm-1:30pm)
- DFC Committee (12:00pm-1:00pm)
- Scientific Session #7 (1:30pm-3:00pm)
- Refreshment Break (3:00pm-3:30pm)
- Silent Auction Pay/Pick-Up (3:00pm-3:30pm)
- SOFT Business Meeting (3:30pm-5:00pm)
- ABFT Certificate Reception (5:00pm-6:00pm)
- Happy Hour (5:00pm-6:00pm)
- President's Reception & Dance (6:00pm-10:00pm)

Friday, October 23, 2015

- Continental Breakfast (7:00am-8:30am)
- Scientific Session #8 (8:00am-10:00am)
- Refreshment Break (10:00am-10:30am)
- Scientific Session #9 (10:30am-12:00pm)
- Closing Ceremony (12:00pm-12:30pm)
- Lunch On Your Own

S01

Analysis of Acetyl Fentanyl in Postmortem Blood and Urine Specimens by Gas Chromatography-Mass Spectrometry

Marissa J. Finkelstein*[1], Chris W. Chronister[1], Christina Stanley[2], Laurie Ogilvie[2] and Bruce A. Goldberger[1]; [1]University of Florida Health Pathology Laboratories – Forensic Toxicology Laboratory, Gainesville, FL, [2]Rhode Island Office of State Medical Examiners, Providence, RI

Background/Introduction: Acetyl fentanyl was first synthesized as an analog of fentanyl in 1960. Its recreational use peaked in 2013. Since the spring of 2013, at least 31cases have been reported in North Carolina, Florida, Rhode Island, West Virginia, Louisiana, and Maryland. Acetyl fentanyl is a μ-opioid agonist, and its pharmacological effects include altered mood, euphoria, respiratory depression, and central nervous system depression. Acetyl fentanyl is used as a substitute for heroin because of its similar pharmacological effects. Due to its structural similarity to fentanyl, acetyl fentanyl exhibits significant cross-reactivity to fentanyl enzyme-linked immunosorbent assays (ELISA).

Objective: The objective of this study was to quantitate acetyl fentanyl in postmortem blood and urine specimens, as well as to investigate the decedent demographic characteristics in order to determine potential factors indicative of acetyl fentanyl use with regards to age, sex, race, and drug use.

Method: Solid-phase extraction (SPEware CEREX® Trace-B) was used to isolate acetyl fentanyl from the biological matrices. The samples were subsequently analyzed by gas chromatography mass spectrometry (GC-MS) in selected ion monitoring mode for quantitative analysis. This method was developed and validated according to standards established by the Scientific Working Group for Forensic Toxicology (SWGTOX). The method exhibited a linear calibration range from 2.5-50 ng/mL. The lower limit of quantitation was determined as the lowest non-zero calibrator, 2.5 ng/mL. The limit of detection was 0.5 ng/mL and 0.75 ng/mL in blood and urine, respectively. The method was used to quantitate acetyl fentanyl in 19 cases obtained from the Rhode Island Office of State Medical Examiners and the University of Florida (UF) Health Pathology Laboratories – Forensic Toxicology Laboratory. Additionally, all cases were subjected to comprehensive postmortem drug screening. Race was categorized as Caucasian, African American, and other. Age at death was divided into six cohorts: < 20, 21-30, 31-40, 41-50, 51-60, and > 61 years of age. Additional toxicology findings were categorized by drug class: opiates/opioids, benzodiazepines, cocaine, cannabinoids, amphetamines, and ethanol. The circumstances surrounding the death of each decedent and the route of administration of acetyl fentanyl were also investigated.

Result: Since 2013, the Rhode Island Office of State Medical Examiners and the UF Health Pathology Laboratories – Forensic Toxicology Laboratory have received 19 cases involving the use of the designer drug acetyl fentanyl. The acetyl fentanyl blood concentrations ranged from 1.2-945 ng/mL. Acetyl fentanyl use was found to be most prevalent among males and individuals aged 21-30 years. Caucasians (18) exhibited the highest rate of acetyl fentanyl use, and additional analytes, apart from acetyl fentanyl, were present in all of the decedents. The additional toxicology findings were as follows: benzodiazepines (6), cocaine (9), opiates (13), amphetamines (1), cannabinoids (6), and ethanol (8). The analysis of circumstances of death indicated eight cases with a prior history of drug abuse, seven decedents with intravenous drug abuse or paraphernalia, and five cases indicated needle punctures.

Conclusion: SPE followed by GC-MS analysis was determined to be a sensitive and specific method for the quantitation of acetyl fentanyl in postmortem blood and urine. A majority of cases investigated indicated concentrations significantly higher than the typical lethal concentration of fentanyl. It was found that males from 21-40 years old (52%), followed by females from 51-60 years old (15%), showed the highest acetyl fentanyl prevalence for the analyzed cases. All of the decedents were Caucasian, except for one 27 year old African American female. In all cases, additional drugs were detected on the comprehensive drug screen, and a majority of the cases had a prior history of drug abuse.

Keywords: Acetyl Fentanyl, GC-MS, Postmortem Toxicology, Method Validation

S02

Direct Quantification of Cannabinoids, Metabolites and Glucuronides in Blood by Disposable Pipette Extraction and Liquid Chromatography-Tandem Mass Spectrometry

Matthew N. Newmeyer*[1,2], Karl B. Scheidweiler[1], Allan J. Barnes[1] and Marilyn A. Huestis[1]; [1]Chemistry and Drug Metabolism, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD, [2]Program in Toxicology, University of Maryland Baltimore, Baltimore, MD

Background/Introduction: Cannabis is the most commonly abused drug worldwide. The primary active ingredient, Δ^9-tetrahydrocannabinol (THC), is metabolized to 11-hydroxy-THC (11-OH-THC) and 11-nor-9-carboxy-THC (THCCOOH), with subsequent phase II glucuronidation. Cannabinoids are lipophilic and undergo prolonged excretion following chronic frequent intake, making identification of recent cannabis intake difficult. We previously found THC-glucuronide and minor plant cannabinoids, cannabidiol (CBD) and cannabinol (CBN), in blood for up to 0.6, 0.5, and 2.1 h, respectively, after initiation of cannabis smoking, suggesting applicability for identifying recent intake. Similarly, cannabigerol (CBG), Δ^9-tetrahydrocannabivarin (THCV), and the metabolite 11-nor-9-carboxy-THCV (THCVCOOH) might provide additional markers of recent intake. Sensitive and comprehensive methods for quantification of multiple cannabinoids in blood are needed to improve interpretation of results. Disposable pipette extraction (DPX) tips offer a novel, automatable technique for extracting cannabinoids from blood.

Objective: To develop a comprehensive, sensitive blood cannabinoid quantification method for THC, 11-OH-THC, THCCOOH, CBD, CBN, CBG, THCV, THCVCOOH, THC-glucuronide and THCCOOH-glucuronide.

Method: 200 µL blood was fortified with d_3-THC, d_3-11-OH-THC, d_9-THCCOOH, d_3-CBD, d_3-CBN, and d_3-THCCOOH-glucuronide internal standards. Proteins were precipitated with 500 µL acetonitrile followed by centrifugation at 15,000g, 4°C for 5 min. 550 µL supernatant was transferred to a clean microcentrifuge tube containing 200 µL 5% aqueous formic acid. The solution was aspirated 4 times through WAX-S tips (DPX Labs, Columbia, SC, 1 mL tip containing 20 mg resin and 40 mg salt). 90 µL upper, organic layer was transferred to a clean microcentrifuge tube containing 210 µL mobile phase A before centrifugation at 15,000g, 4°C for 5 min. The resulting supernatant was transferred to an autosampler vial. Gradient elution was performed with 10 mM ammonium acetate in water (A) and 15% methanol in acetonitrile (B) at 0.5 mL/min on a Kinetex C18 column (Phenomenex Inc, Torrance CA, 2.1 mm x 50 mm; 2.6 um) with 33 µL injection volume. Autosampler and column oven temperatures were 4 and 40°C, respectively. Two MRMs were monitored for each analyte via negative mode electrospray ionization with scheduled MRM detection on a 5500 QTRAP instrument (Sciex, Framingham, MA). Ion ratios were required to be within ±20% of the calibrators' mean ratio.

Result: Linear ranges were 0.5-100 µg/L for THC and THCCOOH, 0.5-50 µg/L for 11-OH-THC, CBD, CBN, and THC-glucuronide, 1-100 µg/L for CBG, THCV, and THCVCOOH, and 5-500 µg/L for THCCOOH-glucuronide (r^2 > 0.995). Inter-day analytical recovery (bias) and imprecision (N=25) were 95.1-114.0% of target and 2.9-8.0% relative standard deviation, respectively. Extraction efficiencies and matrix effects (N=10) were 63.4-118.4% and -50.1 to -2.2%, respectively (N=10). All analytes were stable (±20% of target) after 72 h at 4°C on the autosampler, 16 h at room temperature (except THCCOOH-glucuronide), 72 h at 4°C and three freeze-thaw cycles (N=3).

Conclusion/Discussion: We present a novel, fully validated LC-MS/MS method for quantifying THC, 11-OH-THC, THCCOOH, CBD, CBN, CBG, THCV, THCVCOOH, THC-glucuronide and THCCOOH-glucuronide in blood. This is the most comprehensive blood cannabinoids method to-date employing automatable DPX extraction achieving 0.5-5.0 µg/L lower limits of quantification. This method can be applied for clinical and forensic testing and during clinical research studies investigating CBG, THCV and THCVCOOH for identifying recent cannabis intake.

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

Keywords: Cannabinoids, Blood, Disposable Pipette Extraction

S03
Tissue Distribution of Lamotrigine in Postmortem Cases

Erin Ehrlinger[*1], Rebecca Jufer Phipps[1], Cherrelle Dugger[2], Barry Levine[1] and David Fowler[1]; [1]Office of the Chief Medical Examiner, State of Maryland, Baltimore, MD, [2]Virginia Commonwealth University, Richmond, VA

Introduction: Lamotrigine (Lamictal®) is a dichlorophenyltriazine derivative that was approved for use in the US in 1994 as an adjunct therapy for the treatment of epilepsy in. Lamotrigine has since been used for the treatment of partial and generalized tonic-clonic seizures, as a monotherapy or adjunct therapy and for maintenance treatment of bipolar I disorder. Lamotrigine's mechanism of action is a result of inhibition of excitatory neurotransmitters and blockade of voltage-dependent sodium channels. Following oral administration of an immediate release formulation, lamotrigine has a bioavailability of 98% and ~55% of the drug is bound to plasma proteins. Therapeutic concentrations in blood can range from 2 – 14 mg/L, depending on dosage and type of treatment. Lamotrigine is eliminated largely as glucuronide conjugates with a half-life of ~15-30 hours, which may be altered in the presence of other drugs, particularly enzyme inducing drugs or valproic acid. The Office of the Chief Medical Examiner, State of Maryland has seen approximately a 10-fold increase in lamotrigine positive postmortem cases over the past 10 years, possibly due to the increasing use of lamotrigine in psychiatric disorders.

Objective: To develop a method for the quantitation of lamotrigine in postmortem specimens using SWGTOX guidelines and to evaluate the distribution of lamotrigine in postmortem cases.

Method: Lamotrigine was quantitated using liquid-liquid extraction with a solid support followed by gas chromatography-mass spectrometry. Case specimens (1 mL fluid or tissue homogenate) were spiked with an internal standard (d7-meprobamate), buffered to pH 7 and applied to Chem Elut® columns, eluted with methylene chloride, and evaporated to dryness. The residue was reconstituted with methanol and injected into a gas chromatograph equipped with a HP-5 analytical column (25 m x 0.32 mm x 0.17 µm) and a mass selective detector operated in the SIM mode. A calibration curve using 4 calibrators ranging from 1.0 to 8.0 mg/L was used for quantitation.

Result: In a review of lamotrigine positive cases from 2010 through current cases, medical examiners cited cause of death as lamotrigine intoxication or mixed drug intoxication (in which lamotrigine was specified) in a total of 14 cases. In all of these cases, the concentration of lamotrigine was greater than the published therapeutic range of 2 – 14 mg/L. For the non-lamotrigine related deaths (n=135), the mean blood concentration was 3.7 mg/L (range: 1.0 – 17 mg/L) and the mean liver concentration was 28 mg/kg (range: 1.0 – 87 mg/kg). In lamotrigine-related deaths (n=14), the mean blood concentration was 38 mg/L (range: 20 – 79 mg/L) and the mean liver concentration was 208 mg/kg (range: 100 – 396 mg/kg). Liver to blood (L/B) ratios were calculated, where possible, for non-lamotrigine related deaths and lamotrigine related deaths. Data showed that the L/B ratios were similar in both groups, with a mean ratio of 5.0 (0.95 – 11; n=9) for non-lamotrigine related deaths and a mean ratio of 6.3 (3.6 – 8.3; n=8) for lamotrigine related deaths. To further investigate the distribution of lamotrigine, multiple specimens from 2015 lamotrigine-positive cases were analyzed. Lamotrigine concentrations for these cases are provided in **Table I**.

Table I: Distribution of Lamotrigine (mg/L or mg/kg) in Postmortem Specimens from 2015

Case #	Cardiac Blood	Peripheral Blood (F)	Urine	Bile	Liver	Kidney	Vitreous Humor
1	13		25		147	6.1	2.1
2	1.7	2.0	<1.0	7.0	19	2.3	<1.0
3	60	41	21	34	396	20	8.8
4	7.6		22	50	18	14	4.9
5	6.9		9.1	20	26		2.2
6	4.0	4.8	18	56	43	15	3.4
7					39		
8	8.4*			9.7	3.2		<1.0
9	4.1		9.5		9.0	13	1.3

*Cavity Blood; F = femoral

Conclusion: In the limited number of cases summarized above, postmortem lamotrigine blood concentrations do not appear have significant site-dependent differences. Liver concentrations were an average of 5-6 times greater than blood concentrations. Bile contained relatively high concentrations, making it a suitable postmortem specimen to screen for lamotrigine use. With the increase in lamotrigine positive cases as well as additional indications for treatment with lamotrigine, this evaluation of postmortem lamotrigine distribution will provide toxicologists and medical examiners with additional data to consider when determining cause and manner of death in positive cases.

Keywords: Lamotrigine, Postmortem, GC-MS

S04

Comparing Smoked and Vaporized Cannabinoid Disposition in Blood and Oral Fluid

Rebecca L. Hartman*[1], Nathalie A. Desrosiers[2], Timothy L. Brown[3], Gary Milavetz[4], Andrew Spurgin[4], David A. Gorelick[5], Gary Gaffney[6] and Marilyn A. Huestis[1]; [1]Chemistry and Drug Metabolism, Intramural Research Program, National Institute on Drug Abuse, NIH, Baltimore, MD, [2]Toxicology Section, New York State Police Forensic Investigation Center, Albany, NY, [3]National Advanced Driving Simulator, University of Iowa, Iowa City, IA, [4]College of Pharmacy, University of Iowa, Iowa City, IA, [5]Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD, [6]Carver College of Medicine, University of Iowa, Iowa City, IA

Background: Although smoking is the most common cannabis administration route, increased medical and legal cannabis intake is accompanied by greater vaporization use to reduce harmful byproduct exposure. Lower vaporization heating temperatures release less THC, but vapor is contained, reducing sidestream loss versus smoking. Limited data exist comparing vaporized to smoked cannabinoids.

Objective: To compare vaporized to smoked blood and oral fluid (OF) cannabinoid disposition.

Method: Healthy adults provided informed consent for two different Institutional Review Board-approved protocols. In the first, occasional smokers (<2x/week) smoked one 54mg Δ^9-tetrahydrocannabinol (THC) cigarette *ad libitum*. Blood and OF were collected before and up to 8h post-dose (first post-dose specimens collected at 0.5h). In the second, occasional-to-moderate smokers (≥1x/last 3 months, ≤3 days/week) inhaled vaporized ground bulk cannabis (35mg THC, 210°C) *ad libitum*. Blood and OF were collected before and up to 8.3h post-dose (first post-dose blood and OF specimen collected at 0.42 and 0.17h, respectively). Blood and OF were quantified for THC and 11-nor-9-carboxy-THC (THCCOOH) by published methods. Blood quantification was performed by solid-phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LCMSMS, THC and THCCOOH limits of quantification [LOQ] 1µg/L); OF by SPE and two-dimensional gas chromatography-MS (2D-GCMS, LOQ THC 0.5µg/L and THCCOOH 15ng/L). Observed maximum concentrations (C_{max}) and area under the curve (AUC, linear trapezoidal method) including baseline [BL], 0.5-8h [smoked] and BL, 0.42-8.3h [vaporized] (blood); and BL, 1-8h [smoked] and BL, 1.4-8.3h [vaporized] (OF) were determined for THC and THCCOOH. Earlier post-dose OF collection times (smoked, 0.5h; vaporized, 0.17h) were mismatched, precluding comparison. Cannabinoid pharmacokinetic dispositions were evaluated, and between-group analyses performed by nonparametric Mann-Whitney *U* test.

Result: Eleven volunteers (ages 22-41) participated in the smoking protocol; 19 (ages 21-37) completed the vaporization protocol. See median [range] blood THC and THCCOOH C_{max} and AUC (not dose-normalized) after smoking and vaporization below.

			Smoked	Vaporized	p_{s-v}
Blood (Smoked: BL→0.5→8h; Vaporized: BL→0.42→8.3h)	THC	C_{max} (µg/L)	12.1 [4.1-40.3]	13.2 [2.4-40.8]	0.846
		AUC (h*µg/L)	18.1 [3.0-56.6]	21.7 [1.7-86.7]	0.846
	THCCOOH	C_{max} (µg/L)	10.4 [6.5-27.4]	23.8 [2.6-66.6]	0.089
		AUC (h*µg/L)	46.2 [25.6-103]	89.1 [9.1-365]	0.081
OF (Smoked: BL→1→8h; Vaporized: BL→1.4→8.3h)	THC	C_{max} (µg/L)	238 [48.4-1105]	91.0 [9.3-1028]	0.060
		AUC (h*µg/L)	417 [102-2181]	209 [21.2-2237]	0.116
	THCCOOH	C_{max} (ng/L)	28.5 [0-168]	15.5 [0-414]	0.775
		AUC (h*ng/L)	100 [0-512]	15.5 [0-1898]	0.864

Median smoked and vaporized concentration vs. time curves were similar for blood and for OF, albeit with large intersubject variability. For both administration routes, blood and OF THC observed C_{max} occurred in the first post-dose specimens (no t_{max} difference). Irrespective of inhalation route, participants feel effects almost immediately and can control inhalation to achieve desired subjective and physiological effects.

Conclusion: Smoked and vaporized cannabis produced notably similar cannabinoid disposition, even after different administered doses, participants, time points and smoking histories. Both *ad libitum* inhalation routes allow rapid THC absorption and delivery to brain. A within-subjects study design with the same THC dose could further facilitate comparison; however, despite these limitations, self-titration evidence was clearly observed.

Research supported by NIDA/NIH Intramural Research Program, NHTSA, and ONDCP.

Keywords: Vaporization, Smoking, Cannabis

S05
Buprenorphine in Georgia: A 2 Year Case Review

Jon Stephenson*; Georgia Bureau of Investigation, Division of Forensic Sciences, Atlanta, GA

Introduction: Available since 1985 buprenorphine has increased in use across the United States for its applications in pain management clinics and the controlling of opioid withdrawal. With buprenorphine requests increasing the Georgia Bureau of Investigation developed a method for its analysis which was implemented in December 2012.

Objective: Conduct a 2 year review of all 67 cases containing buprenorphine from the period of December 2012 to December 2014, examining the concomitant consumption of drugs and the impairing effects observed by police officers.

Method: Whole blood samples from individuals suspected of driving under the influence of drugs were analyzed for buprenorphine. Samples were screened by LC-MS/MS for 80 different compounds including buprenorphine using a protein precipitation with acetone. Quantitation was performed by LC-MS/MS using a solid phase extraction method.

Result: All cases analyzed were found to have at least one additional psychotropic drug despite the concerns, particularly with benzodiazepines, associated with combined use of buprenorphine. The percentage of occurrences of drug classes is 84% benzodiazepines, 32% antidepressants, 30% opioids, 26% amphetamines, 24% antihistamines, 18% anticonvulsants, 14% THC, 11% muscle relaxants, 9% antipsychotics, 5% barbiturates, 3% cocaine, 3% over the counter medications, 3% sleep aids, and 1% anesthetics.

Concentrations of buprenorphine and norbuprenorphine in traffic cases were analyzed, norbuprenorphine was reported qualitatively only until March 2014.

	Buprenorphine µg/L (N=59)	Norbuprenorphine µg/L (N=34)
Mean	4.2	4.2
Median	3.1	3.5
Mode	1.3	1.7
Range	0.75-37	0.75-8.3

Table calculations exclude values below the lowest calibrator of 1 µg/L.

Six case histories were examined, with officers commonly reporting constricted pupils, slurred speech, and the subjects being unsteady on their feet. When field sobriety tests were conducted the six subjects commonly demonstrated lack of smooth pursuit, nystagmus at maximum deviation and onset prior to 45° in the horizontal gaze nystagmus test; lack of balance, missing heel-to-toe, stepping off the line, and improper turns in the walk-and-turn test; body sway, putting their foot down, and using their arms for balance in the one-leg-stand test.

Subject	Age	Sex	Buprenorphine (µg/L)	Norbuprenoprhine (µg/L)	Additional Drugs	Admittedly Consumed Drugs
1	40	Female	8.9	3.9	Clonazepam 16 µg/L Alprazolam <12.5 µg/L Paroxetine Hydroxyzine	Suboxone Paxil
2	33	Female	1.2	4.8	Clonazepam 240 µg/L	Klonopin
3	32	Female	8.1	Qualitative	Alprazolam 180 µg/L Oxycodone 28 µg/L Tramadol 1500 µg/L Fluoxetine	Xanax
4	36	Male	2.2	3.3	Alprazolam 22 µg/L	Xanax
5	62	Male	1.6	Qualitative	Alprazolam 77 µg/L	Suboxone
6	22	Female	1.3	Qualitative	Clonazepam 13 µg/L	Klonopin
6 (again)	22	Female	4.3	Qualitative	Clonazepam 59 µg/L	Klonopin

Conclusion: Given the sample population analyzed for this study (suspected DUI drivers) it is apparent that buprenorphine combined with other psychotropic drugs can lead to impairment even at therapeutic doses of both drugs. For buprenorphine related DUI cases in Georgia the most commonly consumed class of drugs were benzodiazepines, with no single case examined containing only buprenorphine. This adds to the ever growing evidence demonstrating the potentiation of buprenorphine with concurrent use of benzodiazepines or other psychotropic drugs increasing the likelihood of impairment.

Keywords: Buprenorphine, Opioid, Driving

S06

Comparison of On-Site and Laboratory Based Techniques for the Analysis of Drugs in Oral Fluid

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Introduction: This study used the Alere DDS®2 handheld rapid oral fluid screening device to detect drugs in oral fluid of voluntary subjects encountered on patrol in Tulsa, OK. Some of the subjects were also evaluated by drug recognition experts (DREs). Oral fluid samples were collected using a Quantisal™ device for archival purposes and were tested via ELISA and LC-MS/MS.

Objective: In this study, the objective was to determine whether an oral fluid rapid screening device would provide comparable results to laboratory based screening and confirmation testing.

Method: Oral fluid specimens were collected from forty-two individuals for this study. Of these samples, five were collected during DRE training and thus a DRE opinion was provided. These 5 specimens also had results from a urine drug screen and any medications self reported by the individual. Oral fluid was analyzed onsite using rapid screening technology (Alere DDS®2) for six classes of drugs (amphetamine, benzodiazepines, cocaine, methamphetamine, opiates, and THC). An archival Quantisal™ sample was also collected for laboratory based immunoassay (ELISA) screening, which looked at the same six classes of drugs. Quantisal™ samples were confirmed by LC-MS/MS.

Result: The trueness of the DDS®2 was assessed using the LC-MS/MS confirmation result. ELISA correlation will also be presented.

	COC	OPI	BZD	THC	AMP	METH
True Positive	1	3	1	11	12	9
True Negative	41	39	41	31	25	32
False Positive	0	0	0	0	5	1
False Negative	0	0	0	0	0	0
Sensitivity (%)	100	100	100	100	100	100
Specificity (%)	100	100	100	100	83.33	96.97
PPV (%)	100	100	100	100	70.59	90
NPV (%)	100	100	100	100	100	100
Accuracy (%)	100	100	100	100	88.10	97.62

Subject	DRE Opinion	Oral Fluid DDS®2	Oral Fluid ELISA	Oral Fluid LC-MS/MS	Urine Screen
1	Depressant & Stimulant	AMP	BZD, AMP	BZD, AMP	BZD, AMP, PCP
2	Depressant & Stimulant	AMP	BZD, AMP	BZD, AMP	BZD, AMP, PCP
3	Cannabis	Not Detected	Not Detected	Not Detected	THC
4	Depressant, Stimulant, & Cannabis	AMP, METH	COC, BZD, THC, AMP, METH	COC, BZD, AMP, METH	BZD, METH, THC
5	Narcotic, Stimulant, & Cannabis	AMP, METH	BZD, AMP, METH	AMP, METH	AMP, METH, THC, Methadone, Oxy, OPI, COC

Discussion: The results from a rapid oral fluid screening device (DDS®2) correlate well with laboratory based techniques when using the stated cutoff concentrations of the device. Data supports the utility of the DDS®2 to be used onsite to detect recent drug use in individuals.

Keywords: Oral Fluid, Rapid Screening Device, LC-MS/MS

S07

Cannabis and Low-Dose Alcohol Effects on Longitudinal Control in Simulated Driving after Controlled Administration

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Background: Medical and recreational cannabis acceptance is leading to increased frequency of driving under the influence of cannabis. Specific effects of cannabis on driving, with and without commonly co-occurring alcohol, are not fully characterized.

Objective: To evaluate cannabis and low-dose alcohol effects on drivers' longitudinal control (speed, standard deviation [SD] of speed, and headway maintenance relative to lead vehicle).

Method: Current occasional (≥1x/last 3months, ≤3days/week) cannabis smokers provided written informed consent (IRB-approved controlled-administration study). Participants received placebo or low-dose alcohol (median [range] 0.058 [0.034-0.135]g/210L peak breath alcohol concentration [BrAC]) and orally inhaled 500mg placebo, low (2.9%)-Δ^9-tetrahydrocannabinol [THC], or high (6.7%)-THC vaporized cannabis (Volcano® Medic) in separate sessions (6 conditions; randomized, within-subject, placebo-controlled). Participants drove multi-stage simulated drives (National Advanced Driving Simulator, University of Iowa), including urban, interstate, and rural components (~0.8h duration). Blood THC concentrations before (0.17h, 0.42h) and after (1.4h, 2.3h) driving (driving occurred 0.5-1.3h post-dose) were determined by liquid chromatography-mass spectrometry (limit of quantification 1.0µg/L). BrAC at these same times were measured by Intoximeters Alco-Sensor IV. THC concentrations during drives were estimated from fitted individualized power-curve models; BrAC by linear interpolation from pre- and post-drive concentrations. Mean speed (relative to speed limit), SD speed, percent of time spent >10% above or below the speed limit ("percent speed high", "percent speed low"), longitudinal acceleration (forward acceleration/deceleration) and ability to maintain headway relative to a lead vehicle ("headway maintenance") were analyzed vs. blood THC and BrAC for cannabis, alcohol, and cannabis*alcohol interaction (stepwise general linear model select approach), accounting for speed limit and road curvature.

Result: Eighteen participants (13M, 5F, ages 21-38) completed all sessions. Blood THC concentration was significantly associated with decreased mean speed (approximately 0.10mph (0.17km/h) per µg/L, such that 2, 5, and 10µg/L THC decreased speed 0.21, 0.54, and 1.1mph (0.34, 0.87, and 1.7km/h), respectively) and increased percent speed low ($p<0.0001$), but not significantly with SD speed. In contrast, BrAC was significantly associated with increased SD speed (0.05mph (0.08km/h) per 0.01g/210L BrAC, such that 0.02, 0.05, and 0.08g/210L produced 0.10, 0.26, and 0.41mph (0.16, 0.42, 0.66km/h), respectively) and percent speed high ($p<0.0001$), but not mean speed. Neither THC nor BrAC affected longitudinal acceleration. THC increased drivers' average following distance to a lead vehicle; BrAC did not. Only one variable showed a significant THC*BrAC interaction: percent speed high (negative, indicating lessened overall effect of BrAC when combined with THC), but only when considering positive [non-zero] values and excluding an outlying driving event (interstate off-ramp, wherein drivers remained at interstate speed and decelerated to slow near the end).

Conclusion: Alcohol produced faster driving and general decrements in ability to control speed, whereas cannabis decreased speed and increased time spent below the speed limit in a concentration-dependent manner. Drivers under the influence of cannabis may be more aware of potential impairment and attempt to compensate by driving more slowly.

Research was supported by the NIDA/NIH Intramural Research Program, NHTSA, and ONDCP.

Keywords: Cannabis, Alcohol, Driving

S08
Heroin in Wisconsin Drivers

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Background/Introduction: Heroin (diacetylmorphine) abuse is widespread in Wisconsin and may be underestimated in operating while intoxicated (OWI) cases. Heroin, a Schedule I Restricted Controlled Substance (RCS), is rarely confirmed in blood of drivers due to its extremely short half-life (T ½) of 2-6 minutes. Unequivocal evidence of illicit heroin use can be confirmed by the presence of its metabolite, 6-monoacetylomorphine (6-MAM), which is not currently scheduled in Wisconsin. Clandestine production of heroin results in small amounts of codeine as an artifact of the acetylation process. Previous studies indicate that in the absence of 6-MAM confirmation, morphine/codeine ratios (M/C) greater than 1.0 may also indicate heroin use – concentrations of codeine in these cases are minimal, typically less than 10% of reported morphine values. Here we present case histories, driving observations, and full toxicology results for subjects suspected of driving under the influence of heroin as determined by confirmation and quantitation of 6-MAM.

Objective: The objectives of this study are to provide forensic and roadside performance results for traffic cases involving individuals driving while under the influence of heroin and driving cases where M/C is greater than 1.0 as an indicator of heroin use. In addition, the time intervals between the traffic event, collection of the blood, and laboratory testing will be evaluated to determine the effects of turnaround time on confirmation of 6-MAM.

Method: Whole blood was collected from subjects arrested for alleged OWI and sent to the Wisconsin State Laboratory of Hygiene for testing. In all cases, ethanol and comprehensive drug testing was performed. Free opioids were determined by a validated solid phase extraction (SPE) method followed by confirmation and quantitation with gas chromatography mass spectrometry (GC-MS). Case histories, driving evaluations, and demographic information were obtained from police reports when available for all cases where 6-MAM was confirmed.

Result: In opioid analyses from February 2012 to February 2014, morphine was detected in 908 specimens; 253 (28%) contained both morphine and codeine; the M/C ratio was >1.0 in 244 specimens. 6-MAM was confirmed in 8 (<1%) cases. Median concentrations (range) for morphine, codeine and 6-MAM were 260 (83-410), 14 (10-20) and 6.5 (4-30) ng/mL, respectively. Limits of quantitation for this method are 10 ng/mL (morphine and codeine) and 4 ng/mL (6-MAM). For 7 of 8 specimens in which 6-MAM was confirmed, the time between receipt of the specimen and testing for opioids ranged from 31 to 94 days, with an average of 65 days. One specimen had a time interval of 194 days. Subjects ranged in age from 22 to 53 years old. Six of 8 vehicles were involved in an off-road or crash incident and 3 of 8 subjects were evaluated by Drug Recognition (DRE) officers. Indicators of impairment included miosis with minimal to no pupillary response to light, ptosis, lethargy, and poor balance. Ethanol was detected in one subject (0.048 g/dL), and 7 of 8 subjects had drugs other than morphine, codeine, and 6-MAM confirmed. Additional drug findings in these subjects include delta-9-THC and metabolites, diphenhydramine, benzodiazepines, benzoylecognine, quinine, hydrocodone, and oxycodone.

Conclusion/Discussion: Previous studies indicate that M/C >1.0 suggest heroin use. In our study, 244 of 253 Wisconsin drivers under the influence of morphine and codeine had M/C >1.0. Absolute evidence of heroin intake, however, was confirmed by the presence of 6-MAM in only 8 cases. As laboratory turnaround time decreases, we expect 6-MAM confirmations to become more frequent. Subjects driving under the influence of heroin demonstrated significant impairment consistent with narcotic analgesics.

Keywords: Heroin, Diacetylmorphine, 6-MAM

S09

Preliminary Psychophysical Task Performance after Controlled Oral Cannabis Administration

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Background/Introduction: Drug Evaluation and Classification (DEC) examinations categorize physiologic signs of impairment by drug class and are widely implemented in suspected driving under the influence of drugs cases. Cannabis, the most commonly consumed illicit drug worldwide, produces impairing effects on human performance. Cannabinoids are most frequently administered via smoking; however, oral ingestion is also common. Performance on DEC tasks after this administration route is not thoroughly characterized.

Objective: The objective was to characterize cannabis' effects on psychophysical exam performance after controlled oral administration in occasional and frequent cannabis smokers.

Method: In this Institutional Review Board-approved study, occasional (≥2x/month but <3x/week) and frequent (≥5x/week) cannabis smokers participated in a double blind, randomized, crossover, placebo-controlled cannabis administration protocol. Participants consumed ad lib 750 mg oral placebo (0% Δ^9-tetrahydrocannabinol [THC]) or active (6.9% THC) baked in a brownie and performed a series of 3 psychophysical tasks after dosing. In the modified Romberg Balance (Romberg) task, participants estimated the passage of 30sec; primary clues are the participant's bias from 30sec, presence of eyelid tremors, and body sway. In the One Leg Stand (OLS) participants raised one leg off the ground while maintaining balance for 30sec; clues observed during the exam included participants raising their arms, hopping, swaying and putting their foot down. Finally, in the Walk and Turn (WAT) task, participants walked down a line in heel-to-toe fashion for 9 steps, turned in a pre-defined manner, and walked 9 steps back heel-to-toe; clues for this task included actual number of steps taken and if the participant missed heel-to-toe contact, raised their arms, stepped off the line, or stopped walking. For the OLS and WAT the total number of unique clues was calculated. Psychophysical exam scores were compared between placebo and active doses by Wilcoxon matched-pair signed-rank test with $p<0.05$ significance threshold.

Result: Thirteen participants (8 frequent, 5 occasional) completed both sessions. In the Romberg task, occasional smokers produced significantly ($p=0.038$) shorter estimations (median [range]) after active dosing compared to placebo (28 [18-33]sec and 32 [29-38]sec, respectively), but no significant differences were observed in frequent smokers. There was no significant difference in eyelid tremors or body sway between placebo and active doses. For the OLS, significantly ($p=0.023$) more clues were observed after the active dose (1 [0-2]) in the occasional group than after the placebo (0 [0-1]). For the WAT, there was a significant ($p=0.017$) increase in the number of observed clues after active dosing (n=10 tests with 1 observed clue, 7 tests with 2 clues, and 3 tests with 3 clues) compared to placebo (n=9 tests with 1 clue, 4 tests with 2 clues, and 1 test with 3 clues) when the groups were combined.

Conclusion/Discussion: Oral cannabis administration produced significant psychophysical effects on occasional smokers in the Romberg and OLS tasks; a significant effect was observed on the WAT task only when all participants were grouped together. Additional participant data are forthcoming and will increase statistical power for characterizing oral cannabis' effect on psychophysical exam performance in occasional and frequent cannabis smokers.

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

Keywords: Cannabis Administration Routes, Drug Evaluation and Classification (DEC), Human Performance

S10
Case Study: A DUID Case with Flubromazepam and Other Drugs

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Background: The prevalence of benzodiazepines in driving under the influence is well documented. Recent additions to this common class of drugs observed in the state of Georgia are phenazepam, etizolam and for the first time flubromazepam. Flubromazepam (7-bromo-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one) is a designer benzodiazepine structurally and pharmacologically similar to phenazepam.

Objective: Report a case study involving flubromazepam and other drugs to include analysis of the blood specimen and field report of subject manifestations.

Method: The blood specimen was submitted to the GBI-DOFS headquarters laboratory for toxicology analysis. Following a protein precipitation extraction the blood specimen was analyzed in parallel by immunoassay (CEDIA) and an LC/MS/MS screen for approximately 80 drugs. Follow up confirmation extractions were performed using liquid/ liquid extraction for benzodiazepines and analyzed by GC/MS and second protein precipitation extraction followed by LC/MS/MS. Flubromazepam was identified by GC/MS and cross reaction with the CEDIA benzodiazepine immunoassay.

Result/Case Report: A 34 year old male driver was involved in a rear end collision with another car. When officers arrived they found the man slumped over the wheel. In discussions with the officer the subject seemed confused, sleepy, had thick slurred speech and displayed a lack of balance. The officer performed the standard field sobriety tests (HGN, WAT, OLS). Body sway, and a lack of balance were displayed throughout horizontal gaze nystagmus (HGN, six clues) as well as walk-and-turn (WAT, six clues). The subject's lack of balance caused the officer to discontinue the one legged stand (OLS) due to safety concerns. The subject reported taking several medications for schizophrenia, depression and anxiety including Zyprexa, Effexor, Depakote and Buspar as well as "a new medicine he could not think of".

Analysis of the blood specimen by LC/MS/MS and GC/MS confirmed olanzapine and venlafaxine in the blood specimen. An indicative CEDIA result for benzodiazepines but negative LC/MS/MS screen for common benzodiazepines led to the more general liquid/liquid extraction and identification of flubromazepam by GC/MS. No analysis for valproic acid and buspirone was performed.

Discussion: Flubromazepam is expected to have strong benzodiazepine like effects. The subject displayed classic signs of central nervous system depressants indicative of benzodiazepine impairment. Some or all of the manifestations displayed in this case may be resultant from intoxication by flubromazepam. Additionally, the case demonstrates the utility of analyzing case specimens across a variety of analytical techniques.

Keywords: Flubromazepam, DUID, Designer Benzodiazepines

S11

Trends of Benzodiazepines and Z-Drugs in DUID, Sexual Assault, and Postmortem Cases in Orange County, CA from 2010 – 2014

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Introduction: Benzodiazepines and sedative hypnotics are some of the most frequently abused drugs in driving under the influence of drugs (DUID) cases, coroner cases, traffic collisions and drug facilitated sexual assaults (DFSA). In 2010, the Orange County Crime Lab started screening all cases for these drugs and in 2012, 14 benzodiazepines and three sedative hypnotics were routinely quantitated. The drugs included alprazolam, chlordiazepoxide, clonazepam, diazepam, lorazepam, estazolam, flunitrazepam, flurazepam, midazolam, nordiazepam, oxazepam, phenazepam, temazepam, triazolam, zaleplon, zolpidem, and zopiclone.

Objective: Attendees of this presentation will learn about the prevalence of benzodiazepine and sedative hypnotics in cases over a five year time period. The trends include the most prevalent drug combinations and concentration statistics for the different case types. At least one specific casework example will be given for each case type.

Method: *All whole blood samples were extracted using DPX-WAX tips and a Waters* Acquity UPLC coupled to a Waters TQ-S triple quadrupole mass spectrometer. The method was validated by SWGTOX guidelines and presented at the 2013 Annual SOFT Meeting. All samples were extracted twice so average concentrations could be used in this study. Once the quantitation data was collected, each case was examined for other drugs present and case type. Concentration ranges and prevalence data for each drug and drug combination were examined.

Result/Discussion: Of the 31,927 toxicology cases received from 2010 to 2014, 17% were confirmed for benzodiazepines and/or sedative hypnotics. Of the confirmed cases, 20% had more than one of these drugs, with 10 individuals in DUID cases having five benzodiazepines and/or sedative hypnotics detected. Alprazolam was the most prevalent benzodiazepine and zolpidem was the most commonly detected sedative hypnotic for all case types. Combining all case types, the list of most to least prevalent benzodiazepines were nordiazepam, temazepam, diazepam, clonazepam, lorazepam, oxazepam, chlordiazepoxide, midazolam, flurazepam, triazolam, phenazepam, estazolam, and flunitrazepam. Zopiclone was detected in six cases but no zaleplon has been confirmed. Drug combinations within the same drug class were also examined. Alprazolam and zolpidem and alprazolam and diazepam, or one of its metabolites, was the most common. The most prevalent prescription drugs detected with either benzodiazepines or sedative hypnotics were narcotic analgesics. The most common illicit drug was marijuana in combination with benzodiazepines and methamphetamine with zolpidem. The average concentration and concentration ranges varied for each drug; however, there was no significant difference in drug concentration between case types.

Conclusion: It was not surprising that alprazolam and zolpidem were the most prevalent drugs for benzodiazepines and sedative hypnotics, respectively, for all case types. The most prevalent drug combinations, alprazolam with hydrocodone and carisoprodol, were also seen regardless of case type. It was surprising to find that the concentrations ranges were similar between all case types with individual's driving at concentrations that were determined to be contributing to overdoses in PM casework.

Keywords: Benzodiazepines, Sedative Hypnotics, DUID, Postmortem Toxicology, Sexual Assault

S12
Toxicology Findings in Sexual Assault Investigations in Central Virginia

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Background/Introduction: Toxicology testing is often performed in sexual assault investigations to determine whether the victim may have been incapacitated by ethanol and/or drugs. Central Virginia is a demographically diverse region, including urban, suburban, rural and collegiate populations, serviced by a single toxicology laboratory. Law enforcement agencies are asked to collect blood and urine if the victim reports the assault within 24 hours and only urine if between 24 and 120 hours. The laboratory also provides a drug facilitated sexual assault questionnaire to document: the time of suspected drugging, specimen collection, symptoms, and voluntary ethanol, recreational, prescription, or over-the-counter drug use.

Objective: To review drug facilitated sexual assault investigations to ensure appropriate evidence and background information was submitted and to compare toxicology findings to self-reported ethanol and/or drug use.

Method: All sexual assault investigations examined by the Virginia Department of Forensic Science Central Laboratory toxicology section in 2014 were reviewed. Testing provided depended on police request, but generally, samples were tested by headspace gas chromatography for alcohols, ELISA for cocaine, opiates, methamphetamine, MDMA, phencyclidine, barbiturates, benzodiazepines, methadone, carisoprodol/meprobamate, fentanyl, cannabinoids and zolpidem, and gas chromatography mass spectrometry for alkaline-extractable drugs. Specific methodology parameters are available online in the Virginia Department of Forensic Science Toxicology Procedures Manual. Demographic information, self-reported ethanol and drug use, symptoms and toxicology results were extracted from the case files.

Result: Toxicology results were reported for 28 sexual assault investigations. In most cases, law enforcement followed the laboratory's recommendations for specimen collection and completed the provided questionnaire. Blood and urine were submitted for testing in 20 cases, blood only in two and urine only in six. Victims ranged from 15-62 years old, and all but one was female. The duration of time between the assault and specimen collection was evenly distributed: less than 12 h (N=7), between 12 – 24 h (N=7) and greater than 24 h (N=8). Victims primarily reported central nervous system depression, including drowsiness, loss of consciousness, memory loss, and fatigue.

Most victims (N=18) acknowledged ethanol consumption, with approximately one-third engaged in binge drinking (> 5 drinks) on the day of the assault. Few victims admitted recreational drug use, primarily marijuana, and approximately half were using potentially impairing prescription medications, including antidepressants, opiates, benzodiazepines, amphetamine and sedative/hypnotics. Not surprisingly, the less time between voluntary ethanol/drug consumption and specimen collection, the greater likelihood that blood or urine was positive. Ethanol was detectable in blood and/or urine in six cases, each being collected within 19 hours of the alleged assault; in three of these, blood concentrations were significantly elevated (0.165 - 0.291% by weight by volume) 5-12 hours after the assault. Self-administered drugs were more likely to be detected in urine than in blood. THC carboxylic acid was identified in nine cases.

Several drugs were identified in urine samples that were not reported by victim, including chlordiazepoxide, citalopram, doxylamine, dextromethorphan, diphenhydramine, cyclobenzaprine, morphine and 6-acetylmorphine. It is unknown whether these drugs were surreptitiously administered by an assailant.

Conclusion/Discussion: Law enforcement agencies complied with the laboratory's specimen collection recommendations in most cases. As expected, timely reporting was associated with increased drug and alcohol detection. Law enforcement agencies should be encouraged to collect toxicology specimens as soon as possible when drugs or alcohol are suspected.

Consistent with other drug-facilitated sexual assault surveys, ethanol was the most prevalent compound detected in blood or urine, and urine was more useful for drug detection. Unexpected drug findings may have been from surreptitious administration or unreported victim use; investigators should recognize the extended detection windows in urine and self-reported drug use should cover a similar timeframe.

Keywords: Drug Facilitated Sexual Assault, Blood, Ethanol

S13

Revisiting Sexual Assault Cases with a Broader and More Sensitive LC-QTOF-MS Method for the Detection of Benzodiazepine and Z Drugs

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Background: The prevalence of benzodiazepines and "Z" drugs in sexual assault cases have been well documented in the forensic toxicology literature. Detecting the use of these compounds in victims of sexual assault can be useful for investigators as they are powerful central nervous system depressants that can have amnesic, dissociative, hypnotic and/or sedative effects. Lower limits of detection have been recommended to extend the window of detection in these cases as most victims do not report until hours or potentially days after the incident.

The current methodology employed at our laboratory screens and confirms the presence of eight (8) benzodiazepines and zolpidem. Cases are confirmed on a GC/MS following a presumptive positive ELISA screen with cutoff values of 100 and 25 ng/mL for benzodiazepines and zolpidem, respectively. This approach is satisfactory for DUI cases, however, these cutoffs are well above the SOFT Drug Facilitated Sexual Assault Committee recommended values for sexual assault cases. In an attempt to confirm a greater number of benzodiazepines and "Z" drugs with increased sensitivity, a targeted MS/MS LC-QTOF-MS method was developed and validated.

Objective: The purpose of this study was to evaluate the impact of using a newly developed analytical method that confirms the presence of 25 benzodiazepines and 3 "Z" drugs by LC-QTOF-MS on sexual assault cases. All of the urine sexual assault case samples received since 2010 were reanalyzed using this new analytical method and the results were compared to previously reported results to determine how this method would impact the positive rate for these compounds.

Method: Urine samples were enzymatically hydrolyzed before being subjected to a solid phase extraction method. An Agilent 1260 High Performance Liquid Chromatography system coupled to an Agilent 6530 Quadrupole Time of Flight Mass Spectrometer in targeted MS/MS mode was used. Target analytes included: 7-aminoclonazepam, 7-aminoflunitrazepam, alpha-hydroxyalprazolam, alprazolam, bromazepam, chloridazepoxide, clobazam, clonazepam, demoxepam, desalkylflurazepam, diazepam, estazolam, etizolam, flunitrazepam, flurazepam, hydroxyethylflurazepam, hydroxytriazolam, lorazepam, midazolam, nordiazepam, oxazepam, phenazepam, temazepam, triazolam, zaleplon, zolpidem, and zopiclone. A reverse phase gradient on a C18 column separated the analytes of interest (t= 9.5 min). Mass Hunter Qualitative Data Analysis and PCDL manager software were used for the identification of the compounds. The method was validated in accordance with recommended guidelines. Validation parameters evaluated included limit of detection (LOD), interference, ion suppression/enhancement and carryover. Sexual battery case samples were analyzed using this new method and the results were compared to previously reported results.

Result: A targeted MS/MS method was successfully validated following recommended guidelines. Overall the assay demonstrated sensitivity of ≤5 ng/mL for each compound, which is less than the recommended limit of detections. To date, 20 of the 300 submitted sexual assault cases have been reanalyzed using the LC-QTOF-MS method. Two cases that were previously reported negative had the 7-aminoclonazepam detected and one of these cases also had the presence of hydroxyalprazolam detected after reanalysis. In three of the cases, at least one benzodiazepine was initially reported but additional benzodiazepines and/or metabolites were present in the sample after reanalysis. The previously undetected compounds were 7-aminoclonazepam, alprazolam, hydroxyalprazolam, nordiazepam and demoxepam.

Conclusion: A comprehensive and sensitive method using a LC-QTOF-MS for the confirmation of benzodiazepines and "Z" drugs was developed and validated. After employing this new method, the amount of confirmed benzodiazepine and "Z" drugs in reanalyzed sexual assault cases was higher than previously reported.

Keywords: Sexual Assault, Benzodiazepine, Z Drugs, LC-QTOF-MS

S14

Prevalent Drugs, Symptoms, and Scenarios in DFSA Cases in Alabama from 2004-2014

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Introduction: In 2012, there were more than 270,000 rapes or sexual assaults in the United States. Up to 50% involve alcohol and/or drugs in college women. Drug-Facilitated Sexual Assault (DFSA) occurs when a person is subjected to non-consensual sexual acts while incapacitated or unconscious due to the effects of ethanol, drugs, or other intoxicating substances, and is therefore prevented from resisting or unable to consent. These assaults can occur either opportunistically when a person takes advantage of another who is incapacitated or unconscious through voluntary consumption of drugs and/or alcohol, or surreptitiously when a perpetrator covertly gives a victim a substance designed to incapacitate without consent.

Objective: To investigate the most prevalent drugs, symptoms, and scenarios of DFSA cases in Alabama.

Method: We analyzed sexual assault cases for a 10-year span (2004-2014). Blood and urine were routinely analyzed. Drug screening by enzyme immunoassay was performed using either a Tecan Freedom Evo 75 with Immunalysis Reagents or a Randox Evidence Analyzer. Base and acid/neutral screens were conducted by liquid-liquid extractions and GC/MS. Confirmations were performed in-house or by a reference laboratory via liquid-liquid or solid phase extraction followed by GC/MS and or LC/MS/MS analysis. Ethanol was analyzed by headspace gas chromatography. We used our own laboratory information management system (LIMS) BEAST by Porter Lee to mine and filter data. The data were further analyzed with ArcMAP software program to create a geomap of DFSA distribution per county.

Result: Between 2004 and 2014, 169/7565 (2%) sexual assault/rape cases analyzed by Forensic Biology contained biological specimen kits for Toxicology. Jefferson County, which includes Birmingham, contained the greatest number of DFSA cases submitted. 70% of cases were positive for ethanol and/or drugs and 30% were negative. We identified the most prevalent drugs as: ethanol, cannabinoids, alprazolam, amphetamine/methamphetamine, hydrocodone, diazepam/ nordiazepam, tramadol, benzoylecgonine, and methadone. There were three cases reported with gamma-hydroxybutyric acid (GHB), and no cases reported with flunitrazepam (Rohypnol). In 87% of the DFSA cases, the victim reported voluntarily ingesting a drug or alcohol. Ethanol, marijuana, and alprazolam were the most frequent drugs voluntarily used. The most common symptoms were fragmented memory (56%), blackout (46%), vomiting (15%), drowsiness (7%), and loss of muscle control (5%). All victims were female with an average age of 23 (range 12-54). 80% of the victims were Caucasian and 19% were African American. 95% of the suspects were male with an average age of 29 (range 13-59). 60% of the suspects were Caucasian and 36% were African American. The time between incident and sample collection was on average 22 hours (range 1.5 -155).

Conclusion: It was not unexpected to observe ethanol and other central nervous system depressants as the most prevalent drugs in DFSA cases. These drugs cause drowsiness, physical impairment, and memory loss. Likewise, methamphetamine was identified as a common DFSA drug. Methamphetamine reduces inhibitions and increases sexual energy. The low percentage of GHB positives is consistent with a study by ElSohly and Salamone showing an incidence of 4%. The fact that more than 80% of suspects admitted to voluntary alcohol or drug use correlates with statistics showing that DFSA cases are approximately 80% opportunistic and only 20% surreptitious. Fragmentary memory, in which recollection of an event is disjointed or vague, was more common than blackouts, in which a period of time is simply unrecalled or unknown. There were many instances when specimens were not collected in a timely fashion or incorrect specimens were collected. Timely collection and proper specimens give toxicology labs the best opportunity to identify drugs in DFSA cases. These findings can educate law enforcement, healthcare professionals, attorneys, and fellow toxicologists. We are working on methodology utilizing the Q-TOF to improve limits of detection and identify unknowns by accurate mass.

Keywords: Drug-Facilitated Sexual Assault, Fragmented Memory, Blackouts

S15

Identification of Opioids and Other Drugs in Hair by Proteinase Digestion Followed by UPLC-Tandem-HRMS

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Background/Introduction: Opioids are a class of substances that include natural, semi-synthetic, and synthetic alkaloidal agents derived from opium or substances which have morphine-like activity. Commonly encountered opioids include morphine, codeine, heroin, 6-AM, hydromorphone, hydrocodone, oxymorphone, oxycodone, methadone, meperidine, and tramadol. Buprenorphine and fentanyl are two potent synthetic opioids which are usually given at a much lower dose than other opioids. These compounds and their metabolites may be found in the hair of individuals who have been exposed to the drugs. The detection of the chronic use of opioids has become routine for some laboratories that perform hair testing. Establishing the use or exposure to a single dose of drug, or detection of routinely used low dose/high potency opioids is more challenging. The analytical picture is made more complex when investigating a potential DFSA scenario; the target analyte(s) are often not known.

Objective: To develop a method capable of detecting low dose and/or limited exposure to opioids and other drugs in hair.

Method: The hair is subjected to external decontamination by organic and aqueous solvent washing (methylene chloride, water, methanol), and then dried. 20 milligrams of hair are cryoground to fracture the hair matrix and achieve a high surface area. An enzymatic digestion utilizing Proteinase-K/urea/dithiothreitol is then applied which allows for the complete solvation of the hair at pH 8 in 60 minutes. The hair digest is then cleaned up by solid phase extraction (SCX strong cation exchange). The extract is analyzed by ultra-performance liquid chromatography (UPLC) coupled to a high resolution mass spectrometer (Thermo Q-Exactive). Using positive ESI, the system is capable of multiple analysis modes, including full scan (35,000 resolution), SIM and tandem MS (15,000 resolution). The LC column is BEH C18: 2.1x100 mm, 1.7u (Waters). The mobile phase system consists of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Initial conditions are water/acetonitrile at 98/2% respectively. The organic proportion is increased to 90% by 5.5 minutes and held for two minutes. The column is reequilibrated for two minutes for a total run time of approximately ten minutes. This procedure was validated according to SWG-TOX guidelines.

Result: The method is capable of detecting an array of opioids in hair at detection limits between 1 and 10 picograms per milligram (pg/mg). It was observed that other commonly encountered drugs are also detectable by this method, such as benzodiazepines, antidepressants and over the counter drugs.

Conclusion/Discussion: A method capable of detecting drug concentrations in hair associated with single/limited dosing has been developed. The method is currently validated for opioids, but casework has demonstrated the ability to detect many other drugs of forensic interest. The UPLC allows for excellent peak shape and an analysis time of 10 minutes. The mass spectrometer is capable of high resolution full scan, selected ion monitoring, and tandem modes which allow for flexibility in performing qualitative and potentially quantitative examinations. The near-neutral digestion procedure reduces the analyte discrimination issues encountered when performing acid or base digestions. This allows the method to be used for different classes of drugs and opens up the potential for true untargeted screening of hair specimens.

Keywords: Drugs, Hair, Opioids

S16
Concentrations of 1,1-Difluoroethane in Postmortem and Impaired Driving Cases

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Background: 1,1-Difluoroethane (DFE) is a volatile gas with recognized abuse potential. It is commonly encountered in electronic cleaning products such as Dust-Off™. When DFE is inhaled in high concentrations, it can produce effects similar to anesthetic gases such as disorientation, euphoria and loss of consciousness. The effects peak and dissipate rapidly. Although there have been previous publications describing the symptoms of DFE, there are no published reference ranges that relate concentration of DFE to case history. There is also concern among law enforcement agents regarding the length of time that DFE remains detectable following abuse.

Objective: To summarize and compare the ranges of DFE blood concentrations reported in postmortem and driving under the influence (DUI) cases analyzed over a four year period.
To determine the length of time between incident and collection in DUI cases with detectable DFE in blood.

Method: The concentration of DFE in blood was determined by headspace gas chromatography (GC) or GC with mass spectrometry (GC/MS) by a previously described method[1]. The limit of quantitation for DFE in blood by this method is 0.14 mcg/mL. All positive results for DFE in blood that were reported between 1 January 2010 and 17 April 2015 were extracted from the laboratory information management system. Cases were assigned to postmortem or DUI categories based on the following factors: name of test code ordered (DUI or other), words in the name of the client (coroner, examiner, pathology, autopsy, police or patrol) and other information provided on the requisition (postmortem samples submitted, case history, etc.). Cases that could not be assigned to a category were excluded. A total of 478 postmortem and 192 DUI cases were included in the comparison. The paperwork submitted with the DUI cases was reviewed to determine the interval between the incident and collection.

Result: Postmortem cases had a wide range of DFE blood concentrations from 0.16 to 480 mcg/mL. The 99% confidence interval of the postmortem mean was 88 to 111 mcg/mL. DUI cases also had a wide range of DFE blood concentrations (0.16 to 140 mcg/mL), but there was a significant difference between the two populations $t(560) = 18$, $p < 0.001$. The 99% confidence interval of the mean in DUI cases was 10 to 18 mcg/mL. The interval between incident and collection in DUI cases averaged 65 minutes with one case still positive after 200 minutes.

Discussion: Based on the mean of postmortem cases, 100 mcg/mL would seem like a reasonable threshold for predicting whether a case was DUI or postmortem. However, there was one DUI case with a blood DFE concentration greater than 100 mcg/mL. This case had a collection time of only 20 minutes after the incident, and highlights the need to consider case history when interpreting a blood concentration. DFE was detected in blood up to 3 hours after the incident in DUI cases. This is even longer than would be suggested by the elimination half-life of 23 minutes that was reported for human volunteers exposed to sub-symptomatic concentrations of DFE[2]. Therefore, although rapid collection is recommended, it is reasonable to collect a blood sample for DFE analysis in a DUI case for up to 3 hours after the incident, even if the subject is no longer displaying visible signs of impairment.

[1]Blum, L, Keppel, M & Flail, E, 2008, Forensic Toxicology Proceedings, AAFS.
[2] Ernstgard, et al., 2012, *Toxicology Letters 209*:21-29.

Keywords: Difluoroethane, Impairment, Postmortem

S17
Postmortem Analysis of Kratom (Mitragynine) in North Carolina

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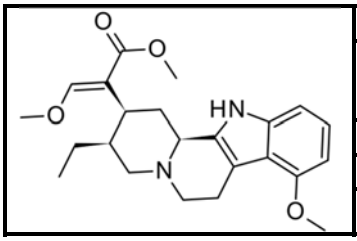
Introduction: Leaves from *Mitragyna speciosa*, known by the common name "kratom," have been chewed by local Southeast Asian populations for medicinal and recreational purposes for centuries. The primary active constituents are mitragynine and 7-hydroxymitragynine, both mu-opioid receptor agonists capable of inducing sedation and respiratory depression, as well as opioid induced constipation and addiction.

Kratom use has migrated to the United States and abroad, where users can purchase ground dried leaves or concentrated extracts. Increased, "non-traditional" use poses a health risk that has not gone unnoticed to the North Carolina OCME toxicology laboratory.

Method: Mitragynine, related alkaloids, and metabolites are first identified by a routine GC/MS organic bases screen (elution order: verapamil, **mitragynine**, trazodone). An LC/MS method was developed for the confirmation of mitragynine and 7-hydroxymitragynine in blood, liver, vitreous humor, and urine using 0.1 mL (g) of specimen. Quantitation is achieved with positive electrospray ionization using a Thermo TSQ Vantage triple quadrupole LC/MS/MS, using LSD-d3 as an internal standard. Two MRM transitions each for mitragynine (399→ 238/174), 7-hydroxymitragynine (415→190/175), and LSD-d3 (327→226/210) are collected, with identification criteria based upon retention time and ion ratios. A whole blood linear calibration curve of 0.050 – 5.0 mg/L, as well as matrix matched controls is included with each batch of specimens.

Result: The laboratory has identified and confirmed mitragynine in 11 postmortem cases. Case study data, including supporting information, causes of death and other drugs involved, will be presented.



	Mitragynine (mg/L)				
	Blood		Liver (ng/g)	Vitreous	Urine
	Central	Peripheral			
Range	0.60-0.60	0.021-3.8	0.48-3.8	0.16	0.05-1.8
Median	0.60	0.27	1.8	0.16	0.92
n =	2	8	5	1	2

Conclusion: Compared to the majority of pharmaceutical and recreational drugs, kratom use is small, but could be easily missed or overlooked in postmortem cases. As potent mu-opioid receptor agonists, the activity of mitragynine and 7-hydroxymitragynine should not be discounted, particularly in cases involving other CNS depressants, such as other opioids or benzodiazepines.

Keywords: Kratom, Mitragynine, Postmortem, LC/MS/MS

S18
Illicit Fentanyl-Related Fatalities in Florida: Toxicological Findings

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Introduction: Fentanyl is a potent opioid prescribed for relieving pain and inducing anesthesia, and has a high potential for abuse. A surge in fentanyl-related fatalities has been periodically reported throughout the United States; the outbreak in 2005-2007 was particularly serious. The UF Health Pathology Laboratories-Forensic Toxicology Laboratory observed a resurgence of fentanyl-related deaths starting in mid-2014.

Objective: This study examines toxicological findings, demographics of the decedents, and circumstances of death in the recent fentanyl-related fatalities, which occurred in Florida. The prevalence of concurrently detected drugs, particularly 6-acetylmorphine, morphine and cocaine was evaluated. The attendees will be informed of the fentanyl concentration range in these postmortem cases and also potential risk factors and comorbidity associated with such deaths.

Method: The postmortem cases submitted to the UF Forensic Toxicology Laboratory for toxicological analysis from July, 2014 to January, 2015 and tested for fentanyl in blood specimens following urine/bile drug screens were included. The specimens for this study were obtained by the Florida district medical examiners. Ninety percent of the blood specimens were collected from peripheral sites with the remaining portion from heart. Fentanyl was isolated from biological matrix via mixed-mode solid phase extraction and subsequently analyzed utilizing gas chromatography-mass spectrometry. The limit of detection (LOD) was 0.62 ng/mL, the limit of quantification (LOQ) was 2.5 ng/mL and range of linearity was 2.5-50 ng/mL.

Result: During the seven month period, the laboratory tested 143 cases for fentanyl. Of those, 72 cases (50%) had quantifiable fentanyl in postmortem blood. The fentanyl blood concentrations ranged from 2.5-68 ng/mL, with a median of 9.8 ng/mL; 6 cases had a concentration >LOD but <LOQ. The decedents were 19-78 years of age (median 41.5 years), 71% male and 93% White (6% Black and 1% Hispanic). Of the 72 fentanyl-related deaths, 71% were drug intoxication fatalities, 14% non-drug intoxication fatalities and 15% pending. Heroin use was often suspected in the cases (44%), more so than fentanyl (36%). Toxicology testing revealed that 4, 42 and 39% of the cases were concurrently positive for 6-acetylmorphine, morphine and cocaine in blood, respectively; 39% were positive for 6-acetylmorphine in urine. Other opioids and benzodiazepines were found in 24 and 40% of the postmortem blood specimens, respectively. Acetyl fentanyl was identified in four cases (>LOD – 10 ng/mL in blood). Seven antemortem blood specimens were submitted; five had fentanyl concentrations of >LOD – 17 ng/mL, one <LOD, and one positive but insufficient sample volume for quantification. For 52 of the 72 cases positive for fentanyl in postmortem blood, the death occurred in District 12 (Sarasota County, n=12; Manatee County, n=40); in 7 additional cases, fentanyl was found in antemortem blood or liver. Fentanyl concentrations in the postmortem blood specimens (all peripheral) were 2.5-50 ng/mL (median 9.5 ng/mL, n=47). Of the overall 59 deaths from District 12, the cause of death was accidental drug intoxication with fentanyl as a sole or contributing factor for 47 cases (2 non-drug intoxication deaths and 10 pending cases). The median fentanyl concentration in postmortem blood was 9.9 ng/mL (range 3.4-50 ng/mL). The median age of the 47 decedents was 34 (range 19-63) years. Males represented 70% of the deaths and 96% were Whites. Most decedents (n=48) did not have a prescription for fentanyl.

Conclusion: A large percentage of the fentanyl-related fatalities included decedents with a history of heroin and intravenous drug use. Concurrent detection of 6-acetylmorphine, morphine, and/or cocaine was frequent. Considering fentanyl's high potency and abuse liability, the recent rise in fentanyl-related deaths is an important public health concern and signifies the urgent need to promote awareness and to establish prevention and treatment efforts.

Keywords: Fentanyl, Postmortem Toxicology, Drug-Related Death

S19

Postmortem Concentrations of Morphine, Fentanyl, Norfentanyl, Acetyl Fentanyl and Noracetyl Fentanyl in Recent Heroin-Related Fatalities

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Introduction: In Hillsborough County, Florida, there were three or fewer heroin-related fatalities per year from 2008 to 2013. However, in 2014, there were 22 heroin-related fatalities and so far in 2015 there have been 18 heroin-related fatalities. Since November 2014, several of the heroin cases involve heroin that has been laced with fentanyl and/or acetyl fentanyl. The case histories and concentrations of morphine, fentanyl, norfentanyl, acetyl fentanyl and noracetyl fentanyl are presented in the heroin-related fatalities identified from November 2014 to present.

Method: Routine drug screening was performed for volatiles by headspace gas chromatography, drugs of abuse by immunoassay (acetaminophen, barbiturates, benzodiazepines, cannabinoids, carisoprodol/meprobamate, benzoylecgonine, fentanyl, methadone, methamphetamine/MDMA, opiates, oxycodone, and salicylates) and alkaline extractable drugs by gas chromatography mass spectrometry (GCMS). In all heroin cases, the immunoassay screens were presumptive positive for opiates and subsequent targeted analyses for unconjugated and conjugated opiates were conducted by LC-MS/MS. In all fentanyl and acetyl fentanyl cases, the immunoassay screens were presumptive positive for fentanyl. Fentanyl and acetyl fentanyl were also detected by looking for their respective ions using full scan GCMS analysis of alkaline extracts. Targeted quantitative analysis of fentanyl, norfentanyl, acetyl fentanyl and noracetyl fentanyl in all available postmortem tissues was conducted by LC-MS/MS.

Result: The victims ages ranged from 22 to 53 years old, 18 were male and 4 were female. All but one had a history of heroin abuse and/or evidence of IV drug use at the death scene such as powders, needles, syringes, tourniquets and fresh track marks. The following table summarizes averages and ranges (parentheses) of drugs concentrations (ng/mL) in blood of the different groups of cases, those with heroin (without fentanyl and acetyl fentanyl, n=9); those with heroin and fentanyl (n=6); those with heroin, fentanyl and acetyl fentanyl (n=3); and those with only acetyl fentanyl (n=4):

Type of case	Morphine (Free)	Morphine (Total)	Fentanyl	Norfentanyl	Acetyl Fentanyl	Noracetyl Fentanyl
Heroin	160 (30-430)	430 (120-1100)				
Heroin and Fentanyl	30 (<20-100)	50 (30-90)	12 (4-27)	1 (0.2-3)		
Heroin, Fentanyl and Acetyl Fentanyl	10 (<20-30)	30 (<20-60)	18 (15-20)	2 (0.8-3)	8 (6-12)	1 (1-2)
Acetyl Fentanyl					467 (310-600)	53 (2-86)

Conclusion: Based on the toxicology results, it is evident that when fentanyl and/or acetyl fentanyl were present, they contributed to the cause of death. In addition, based on the death scenes, high concentrations of fentanyl and acetyl fentanyl and low concentrations of their respective metabolites, it is likely that many of these fatalities occurred very rapidly after drug administration.

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Keywords: Heroin, Fentanyl, Acetyl Fentanyl, Fatalities

S20

Excited Delirium Cases Encountered by the Alabama Department of Forensic Sciences

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Introduction: For many years, the cause of sudden death while in police custody has been a widely debated topic among medical examiners, law enforcement, and the public. In these case types, frequently there is minimal evidence of trauma, but high drug concentrations. Due to the nature of symptoms associated with excited delirium, law enforcement are usually called to restrain the subject where shortly thereafter they expire, spiking the debate as to who is liable for, and what is, the cause of death. Knowledge of common characteristics exhibited by subjects experiencing excited delirium can help law enforcement and emergency medical personnel treat the subject to avoid fatality, but in that event, medical examiners can use these indicators to provide more information into the cause of death.

Objective: To highlight and investigate common characteristics of three potential excited delirium cases received by the Alabama Department of Forensic Sciences.

Method: Routine drug screening by enzyme immunoassay was performed on casework using either a Tecan Freedom Evo 75 with Immunalysis reagents or a Randox Evidence Analyzer. Quantifications were performed in-house or by National Medical Services Labs via liquid-liquid extraction or solid phase extraction followed by GC/MS or LC/MS/MS. Ethanol was quantified in-house using headspace gas chromatography. Medical examiners were consulted to assist with identifying potential cases involving excited delirium.

Result:

Case 1: Police were dispatched to a used car lot where a 30 year old black male was observed breaking in windows of several cars. He was attempting to tear a car apart from the inside when officers attempted to stop him using a taser. When he charged, another police officer deployed his taser. After handcuffing the decedent, his breathing became labored, and he was transferred to the hospital where he passed. Toxicology was performed on peripheral blood in which the results were: methamphetamine 14,000 ng/mL and amphetamine 540 ng/mL. The medical examiner ruled the manner and cause of death, Accident, Methamphetamine Toxicity, respectively.

Case 2: A 30 year old black male was observed waving his hands and cursing in the parking lot of a police department. The man appeared to be hallucinating and when officers tried to make contact with the man, he charged them. Several tasers were deployed and it required a total of 15 officers to handcuff the subject. After being transferred to the jail, he became unresponsive. The toxicology results on central blood were: ethanol 0.069 g/100 mL, cocaine 330 ng/mL, cocathylene 81 ng/mL, benzoylecgonine 1300 ng/mL. The medical examiner ruled the manner and cause of death, Accident, Complication of Cocaine Toxicity Including Excited Delirium, respectively.

Case 3: Officers were dispatched to an apartment complex where a 20 year old white male was fighting with a friend and breaking into nearby apartments to attack neighbors. After fighting off several neighbors trying to calm him, he stabbed a door multiple times trying to reach a barricaded child then ripped an A/C unit from the wall. It took several officers to subdue him. No toxicology was submitted in this case; however, the suspect admitted to taking "7 hits" of synthetic hallucinogen, 2C-I or "smiles".

Conclusion: Subjects experiencing drug-induced excited delirium will typically exhibit aggression, exceptional strength, and rapid breathing. Hallucinations, hyperthermia, and bizarre behavior are often indicators that excited delirium may be present. In excited delirium cases, recognition of symptoms and training in the proper treatment of a person experiencing this kind of episode can help avoid sudden death in police custody. Our findings are consistent with those in literature noting the involvement of stimulants, such as cocaine and methamphetamine, in excited delirium cases. With the increased media attention on in-custody police deaths, an emphasis on exploring excited delirium as a possible contributor or cause of death should be investigated.

Keywords: Excited Delirium, Cocaine, Methamphetamine, 2C-I.

S21
Death in the Dental Chair

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Introduction: Despite the fact that it is an old drug which was synthesized in 1832, chloral hydrate continues to be used today. In this case report, chloral hydrate, nitrous oxide and hydroxyzine were administered in the dental chair by a pediatric dentist to a 66 pound child during a routine dental procedure for the purpose of causing "conscious sedation." After sedation and at the beginning of dental work the patient became restless, vomited, was pulseless, without breath sounds, and was administered cardiopulmonary resuscitation (CPR). After the third round of CPR she was administered epinephrine, subsequently intubated by EMS, administered fluids and died.

Objective: This incident will highlight that necessary interaction and communication between the forensic toxicologist and the medical examiner must occur, and without it dangerous long reaching consequences may result.

Method/ Result: Routine toxicology screening including a base screen found blood, bile and liver hydroxyzine of less than 0.1 mg/L, 0.37 mg/L and 506 mg/kg, respectively. Blood toxicology submitted to a commercial laboratory showed trichloroethanol of 24 mcg/mL, nitrous oxide of 17 mcg/mL. The medical examiner issued the death certificate cause of death as acute chloral hydrate and nitrous oxide poisoning, and manner of death as accident.

In the ensuing investigations a toxicological review was requested by the Board of Dentistry and the prosecuting attorney. After a detailed review, the toxicologist determined that the quantity of drugs was appropriately administered and that this was not an acute chloral hydrate and nitrous oxide poisoning. The detailed review used comparative literature cases, dosing guidelines and pharmacology showing that the deceased was properly medicated. The details of the toxicological interpretation are presented with a discussion of why this was not an overdose. The medical examiner subsequently changed the cause of death to complications of dental sedation using chloral hydrate, nitrous oxide and hydroxyzine; and the manner of death remaining as accident.

Conclusion: The lack of professional communication between the toxicologist and the medical examiner has the potential for causing confusion and misinterpretation of results. Both the medical examiner and the toxicologist must continually work together to prevent interpreting results which may have long reaching consequences.

Keywords: Chloral Hydrate, Dental, Nitrous Oxide

S22
zzzZolpidem, The Silent Killer

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Background/Introduction: Zolpidem, commonly known as the brand name Ambien, is a powerful and long-lasting sedative-hypnotic primarily prescribed for the treatment of insomnia. Zolpidem activates the neurotransmitter GABA which binds to the GABA receptors in the same location as the benzodiazepines, alprazolam and diazepam. The increased GABA activity triggered by zolpidem inhibits the neuron activity that is associated with insomnia. The ultimate outcome is drowsiness, sedation, and calmness which, in turn, induce a deep, sound sleep. Unfortunately, strange and disturbing side effects have been reported with zolpidem use and include hallucinating, sleepwalking, making and eating food while asleep, sleep-driving, and talking on the phone while asleep. Although these symptoms may be harmless, individuals under the influence of zolpidem are at increased risk of harming themselves or others through their actions and behaviors.

Objective: The purpose of this study is to illustrate several cases in which zolpidem use was implicated in the cause and manner of death of decedents in Miami-Dade County, Florida.

Method: Case samples containing zolpidem from 2005 until present day were evaluated to determine its implication in the cause and manner of death of decedents in Miami-Dade County. Data collected, including case demographics, other drugs present, age, race, and quantitative values of zolpidem if available, was tabulated in order to illustrate its prevalence in Miami, Florida and its contribution to cause and manner of death certification. Post mortem specimens including blood, urine, gastric, and liver were deemed positive by Gas Chromatography- Mass Spectrometry (GC-MS), and if necessary, were quantified utilizing solid phase extraction (SPE) followed GC-Nitrogen Phosphate Detector (GC-NPD) analysis. Also, several cases demonstrating the presence and effect of zolpidem will be illustrated.

Result: Zolpidem has been detected in approximately 150 medical examiner cases in the last 10 years, with the majority of the cases occurring within the last 5 years. The cause and manner of death for these individuals consisted of motor vehicle accidents, natural disease, suicides, and drug overdoses. The suicides varied and included drug overdose, drowning, and violent deaths such as hangings and gunshot wounds. Accidental drug overdoses were mostly polydrug toxicities and included other drugs such as alcohol, opiates, and benzodiazepines. Most decedents positive for zolpidem were white males, age 50 and above (45%), followed by white females age 50 and above (21%). Over the years, an increase in zolpidem use among all users along with a broader age range of use was observed. Suicides constituted the majority of deaths at 44% of zolpidem related fatalities, followed by accidents 33%, naturals at 20%, and < 2% as homicides and those still pending a manner of death.

Conclusion: Although the majority of medical examiner cases in Miami, Florida do not list zolpidem in the cause of death, the question remains whether zolpidem plays a factor in an individual's demise. The bizarre behavioral effects associated with zolpidem use combined with extenuating circumstances in some postmortem cases brings into question whether zolpidem is a silent culprit. For a drug so popular and accessible, zolpidem should remain in the forefront as a drug of concern for law enforcement officers, medicolegal investigators, and medical examiners and should not be dozed on in a death investigation.

Keywords: Zolpidem, Postmortem, Polydrug Toxicity, Toxicology, GC-NPD, GC-MS

S23

Metabolic Profiling of α-PVT, a Novel Stimulant Thiophenyl Analog

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Introduction: Novel psychoactive substances (NPS) are constantly emerging onto the illicit drug market to replace newly scheduled substances and circumvent legislation. Usually, little or no pharmacological or toxicological data are available for NPS when they first emerge, making their identification and interpretation in biological matrices challenging. α-PVT (α-pyrrolidinovalerothiophenone), appearing for the first time in 2012, is the thiophenyl analog of the stimulant α-PVP. In 2014, Takayama et al. identified three α-PVT metabolites *in vitro* using human liver microsomes (HLM). However, full pharmacokinetic profiles are still unavailable.

Objective: To generate a comprehensive metabolic profile of α-PVT by assessing its metabolic stability with HLM, metabolism with human hepatocyte incubation and liquid chromatography-high-resolution mass spectrometry (LC-HRMS), and to evaluate *in silico* metabolism prediction for α-PVT metabolites. Additionally, authentic urine specimens from suspected cases of α-PVT consumption were analyzed.

Method: α-PVT was incubated with pooled HLM for up to 1 h, samples were diluted and injected into the Ultimate 3000™ chromatographic system, Thermo Scientific. Separation was achieved within 20 min on an Accucore™ C_{18} column (Thermo Scientific, 100 x 2.1 mm, 2.6 µm). α-PVT was incubated with pooled human hepatocytes – along with diclofenac to document hepatocyte viability – for 0, 30 and 120 min. Samples were diluted for chromatographic separation on a Synergi™ 4 Hydro-RP column from Phenomenex (150 x 2 mm, 4 µm) within 30 min. All samples were analyzed using a Q-Exactive™ Orbitrap from Thermo Scientific. Full-scan data were acquired in positive-ion mode (*m/z* 100 – 600) and triggered data-dependent tandem mass spectrometry ($ddMS^2$). An inclusion list of predicted metabolite masses generated by MetaSite software (Molecular Discovery) was incorporated into the hepatocyte $ddMS^2$ analysis. Additionally, hepatocyte samples were acquired in all-ion fragmentation (AIF) mode (no quadrupole pre-selection) to identify potential unexpected metabolites. Scans were thoroughly data mined with Compound Discoverer™ (Thermo Scientific), looking for phase I and II metabolites (mass tolerance: 5 ppm, intensity tolerance for isotope search: 30%, mass range: ≥ 100 Da). Urine specimens were extracted using cation-exchange solid-phase extraction cartridges, analyzed on the Q-Exactive in $ddMS^2$ and AIF modes, and processed with Compound Discoverer™.

Result: α-PVT exhibited a 31 min half-life, with an intrinsic 21.9 µL/min/mg clearance. This compound is predicted to be an intermediate-clearance drug with an estimated human 10.2 mL/min/kg hepatic clearance. Seven α-PVT metabolites were identified: M1 (pyrrolidine dihydroxylation), M2 (pyrrolidine carbonylation), M3 (thiophenyl carbonylation), M4 (ketone reduction), M5 (pyrrolidine carbonylation and ketone reduction), M6 (pyrrolidine hydroxylation and ketone reduction) and M7 (pyrrolidine hydroxylation). M5 and M2 eluted 3.2 and 5.3 min respectively after the parent compound, suggesting a 2-carbonylpyrrolidine formation. Findings in authentic urine samples were consistent with hepatocyte metabolic studies, M1 being the most intense metabolite in all cases. α-PVT was always detected at higher signal intensities than any metabolite *in vivo*.

Conclusion: For the first time, a complete metabolic profile of α-PVT is presented. In addition to the parent drug, the three most intense metabolites identified *in vivo*, M1, M3 and M4, may serve as markers for α-PVT intake in biological matrices to assist clinical and forensic investigators.

Supported by the NIH/NIDA Intramural Research Program.

Keywords: α-PVT, Human Hepatocytes, High-Resolution Mass Spectrometry

S24

Analysis and Detection of 25B, 25C and 25I-NBOMe in Rat Hair Using SPE/LC/MSMS

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Introduction: NBOMes are phenethylamine derivatives of the 25C-X series, first mentioned in Ralf Heim's PhD thesis in 2004. These were further developed by David Nichol with the addition of a 2-methoxybenzyl (MeOB) onto the nitrogen (N) of the phenethylamine, hence the term NBOMe. The first mention of recreational abuse appeared in 2010, with NBOMe's now routinely associated with the "club drug" scene. They are administered either in liquid form or on blotters due to their high potency and similarities to LSD. [1] Due to NBOMes' toxicity, adverse effects and fatalities have been reported. To date, analytical methods have been published for blood, urine, vitreous humour, brain, liver, bile and gastric contents; however no hair analysis has been published. [2]

Objective: The objective of this research was to extract 25B-NBOMe, 25C-NBOMe and 25I-NBOMe from rat hair by using a phosphate buffer incubation and SPE clean up method. Additional objectives including assessing any dose response relationship and determining whether the colour of hair affects concentration as seen with other phenethylamines.

Method: Long Evans rats (59) were partially shaved prior to first dose. The rats were shaved along their backs ensuring that both white and black hair was collected separately. Rats were then split into 4 groups, receiving saline, 25B-NBOMe, 25C-NBOMe and 25I-NBOMe respectively. Rats receiving NBOMes were subdivided into dose groups (5-6 rats per dose group), receiving 0.03 mg/kg, 0.1 mg/kg or 0.3 mg/kg. Each rat was dosed for a period of 10 consecutive days before being re-shaved; with white and black hair collected separately for analysis. Hair was washed with water and dichloromethane, cut into small segments, and 40mg was transferred into a 7 mL vial. Next,100 µl of 0.01 µg/mL 25B-NBOMe-D3 was added to the vial along with 2 mL of 0.1 M pH 7.4 phosphate buffer. Vials were sonicated for 60 minutes and incubated 12hrs overnight at 40ºC. Sample vials were then centrifuged at 4000rpm adn the supernatant was transferred to SPE cartridges conditioned with 2 mL MeOH and 1 mL 0.1M pH7.4 phosphate buffer. Cartridges were then washed using 3 mL dH_2O, 1 mL 1M acetic acid and 3 mL MeOH before eluting with 3 mL of DCM/IPA/NH_4 (78:20:2). Eluants were evaporated to dryness with nitrogen before being reconstituted in mobile phase and analysed by LC-MSMS.

Result: All drugs were successfully detected in black hair regardless of dose. Black hair incorporated all drugs to a higher degree than the white hair, with only the white hair from rats receiving the 300 mg/kg dose testing positive. Only the white hair from the 25C-NBOMe and 25I-NBOMe 300 µg/kg rats could be quantitated (4 pg/mg each). The concentration increase in black hair was dose-dependent, (2-30 pg/mg). 25I-NBOMe incorporated into the hair the greatest extent, approximately 80% more than 25C-NBOMe and 250% more than 25B-NBOMe.

Conclusion: The use of SPE followed by LC-MSMS analysis allowed for the detection of NBOMes in rat hair. To our knowledge this is the first example of NBOMes being successfully analysed in hair samples, despite the low concentrations administered.

[1] Heim, R., *DescriptionSynthesis and Pharmacology of potent 5-HT 2A receptor agonists Which have a partial N-2-methoxybenzyl structure*, in *Department of Biology, Chemistry and Pharmacy*. 2004, Freie Universitat: Berlin. p. 309.

[2] Poklis, J.L., et al., *Postmortem detection of 25I-NBOMe [2-(4-iodo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine] in fluids and tissues determined by high performance liquid chromatography with tandem mass spectrometry from a traumatic death.* Forensic Science International, 2014. **234**(0): p. e14-e20.

Keywords: NBOMe, Hair Testing, LC/MSMS

S25

Insane in the NBOMe Brain: The Detection of NBOMe Derivatives in Brain Specimens Using LC-Ion Trap MS/MS

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Background/Introduction: N-methoxybenzyl-methoxyphenylethylamine **(**NBOMe) compounds are synthetic psychedelic hallucinogens, commonly sold in powder or blotter-paper form. There are several derivatives, the most popular being 25I-NBOMe (4-iodo-2,5-dimethoxy-N-(2-methoxybenzyl)-phenylethylamine) and 25C-NBOMe (2-(4-cloro-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine). These derivatives, similar to lysergic acid diethylamide (LSD), are agonists for the serotonin 5-HT2A receptor. Stimulation of this receptor triggers desired euphoric and hallucinogenic effects. The abuse of NBOMe derivatives has been suspected in several cases at the Miami-Dade County Medical Examiner Department (MDME) since 2012. However, these derivatives have gone undetected using routine drug screen methods via Gas Chromatography-Mass Spectrometry (GC-MS).

Objective: The objective of this work was to introduce the toxicology community to the theory that in postmortem analysis, brain tissue is a viable specimen for the detection of NBOMe derivatives.

Method: Brain specimens were prepared by homogenizing the brain tissue with DI water, at a 1:1 ratio, by weight. 2 mL of the brain homogenate were then submitted to a solid-phase extraction procedure using mixed-mode United Chemical Technologies (UCT) CleanScreen® Silica columns, and a positive pressure manifold. The NBOMe derivatives were then detected using a Thermo Scientific Dionex UltiMate 3000 Ultra High Performance Liquid Chromatograph (UHPLC), coupled to a Bruker AmaZon SL Ion Trap Mass Spectrometer (MS). A current SWGTOX validated method, targeting designer stimulants including 25I-NBOMe and 25C-NBOMe, was utilized. This method contains an embedded precursor list, which triggers MS/MS analysis on the targeted derivatives in question, if present in the specimen.

Result: Both 25I-NBOMe and 25C-NBOMe were detected in the brain tissue of several cases at the MDME. These cases were chosen for analysis based on case history and per suggestion from the forensic pathologist who performed the autopsy. The detection of these derivatives was confirmed using an in-house library, created from certified reference standards, as well as retention time, parent ion and daughter ion spectra. Three of the cases, where 25I-NBOMe was detected, dated back to 2012 and had an unknown cause of death. A fourth case, dating to 2013, was positive for 25C-NBOMe.

Conclusion: This is the first time NBOMe derivatives have been detected in any cases in the toxicology laboratory at the MDME. To date, the brain tissue from four cases have been positive for either 25I-NBOMe or 25C-NBOMe. Additional specimens from these four cases were analyzed including antemortem serum, postmortem blood, urine and liver, all with negative results. Due to these findings, it is recommended that in postmortem cases where NBOMe use is suspected, brain tissue should be the chosen specimen for analysis.

Keywords: 25I-NBOMe, 25C-NBOMe, Postmortem, LC-Ion Trap MS/MS Screening

S26

Increases in Cases of Severe Toxicity and Deaths Following Use of Synthetic Cannabinoid Containing Products

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Background: SC-containing products supplied by illicit manufacturers were then marketed throughout Europe as herbal incense, before arriving in the United States in November 2008. The prevalence and variety of SCs on the illicit market have steadily increased over the past 6 years, as manufacturers and distributors of SCs and dealers of SC-containing products have attempted to circumvent federal, state, and local laws.

Objective/Method: After being shipped to the United States, the psychoactive substances are typically either mixed with plant material, dissolved in liquid and then applied to plant material, dissolved in liquid for use in e-cigarettes, or dissolved in liquid that users can ingest or mix with another substance (such as energy drinks or tobacco) and consume. Despite warnings ("not for human consumption") and reassurances ("does not contain [any regulated] compounds") on packages, widespread recreational use of these products by a broad demographic, but particularly by younger and inexperienced users, has led to multiple clusters of cases of adverse health effects and deaths. Analysis of biological samples resulting from many of these clusters of illness has demonstrated the various substances responsible for these outbreaks.

Result: SC use has repeatedly been reported to produce serious adverse health effects, including but not limited to excited delirium, acute kidney injury, seizures, psychosis, hallucinations, cardiotoxic effects, coma, and death — with some users dying before they could reach an emergency department. Unlike opioid drugs such as heroin and morphine, SCs have no available antidote, and treatment of the often unpredictable and severe adverse health effects is largely supportive. Increases in clusters of illness involving synthetic cannabinoids have increased steadily since 2012. Clusters of cases identified included at least 2 incidents in each of 2012 and 2013 respectively. Throughout 2014, at least 9 clusters of illness were reported involving a range of synthetic cannabinoids. From January through April, 2015, an additional 9 clusters of illness ranging across the United States have been reported. Additional reported cases of severe toxicity, including death, have increased since 2012.

Discussion: An increase in the incidence of clusters of SC intoxication resulting in severe illness and death has been observed recently. Possible explanations for this trend include better reporting of suspected clusters by health care facilities and local public health entities, enhanced media attention to and reporting of recreational-drug-associated clusters of illness, and improved collaboration between public health and law-enforcement agencies. There is an extended period of time that may elapse before confirmatory testing for new compounds encountered on the illicit market become available, while clandestine illicit laboratories move on to newer compounds. As a result, cases of SC intoxication, fatalities from SC misuse, and outbreaks of severe illness associated with new or particularly toxic compounds are most likely under-recognized. Collaboration between forensic and toxicology laboratories and legitimate suppliers of analytical standards may result in better preparation and a more timely response to future outbreaks. Increased recognition and reporting by clinicians and public health personnel may aid federal and state regulatory efforts in combating this ongoing SC epidemic.

Keywords: Synthetic, Cannabinoid, Enforcement

S27

Pharmacodynamics and Pharmacokinetics of Methylone in Rats

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Introduction: Methylone is a commonly abused synthetic cathinone marketed as a "legal" alternative to "ecstasy" or cocaine. Previous *in vitro* and *in vivo* studies identified 4-hydroxy-3-methoxymethcathinone (HMMC) as the primary metabolite, with other reported minor metabolites, 3,4-methylenedioxycathinone (MDC) and 3,4-dihydroxymethcathinone (HHMC); however, limited pharmacokinetic data are available.

Objective: To develop and validate a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the simultaneous quantification of methylone, HMMC, MDC and HHMC in rat and human plasma. In addition, evaluate pharmacodynamic effects and methylone and metabolites' pharmacokinetics in rats administered 3, 6 and 12mg/kg (human-relevant doses) subcutaneous (s.c.) methylone.

Method: Locomotor activity and temperature were monitored 15, 30, 60, 120, 240, and 480min after s.c. methylone administration to Sprague-Dawley rats. To assess locomotor activity, animals were attributed a single score for a set of behaviors including ambulating, rearing, and sniffing. Methylone and metabolites were quantified in rat plasma specimens (100µL) at the same time points as locomotor activity. Plasma specimens were analyzed by LC-MS/MS on a Synergi polar column with linear ranges of 0.5-1,000µg/L (methylone, HMMC and MDC) and 10-1,000µg/L (HHMC) following β-glucuronidase hydrolysis, perchloric acid protein precipitation and cation exchange SPE. Bias, imprecision, extraction efficiencies and ion suppression were acceptable, except for HHMC, which exhibited higher variability (16.2-37%), low extraction efficiency (15.9-16.2%) and was considered semi-quantitative. The method was cross-validated for human and rat plasma.

Result: Locomotor activity increased post-dosing, with maxima reached within 15-60min. In general, peak behavior included sniffing continuously for 10s and either ambulating or rearing (Score 6). Maximum concentrations (C_{max}) for methylone and metabolites increased proportionally with administered doses, demonstrating linear pharmacokinetics. Methylone exhibited the highest C_{max} at all doses (60-3,168µg/L, coinciding with reported human concentrations) and HMMC the highest AUC (32,328-110,372µg/L), being the predominant metabolite. Time to methylone C_{max} (T_{max}) was 15min, followed by MDC T_{max} 30-45min, with later peak HHMC and HMMC concentrations (T_{max} 55-120min). Methylone and metabolite plasma concentrations correlated positively with locomotor activity scores, with methylone and MDC concentrations exhibiting strong correlations (r=0.64, 0.72, $p<0.0001$, respectively). No significant correlations were observed between temperature and plasma methylone or metabolite concentrations. These results suggest that methylone and MDC mediate locomotor activity after systemic methylone administration, while other metabolites may be inactive or may not pass the blood-brain-barrier.

Conclusion: Methylone is one of the most commonly used synthetic cathinones in the United States despite scheduling efforts. A sensitive and specific LC-MS/MS method for simultaneous quantification of methylone and metabolites, HMMC, MDC and HHMC, in plasma was developed and validated. Pharmacodynamic and pharmacokinetic data for methylone and metabolites can aid in the interpretation of results in forensic and clinical settings, and suggest that methylone and MDC are the primary mediators of methylone toxicity.

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

Keywords: Methylone, 4-Hydroxy-3-methoxymethcathinone, Novel Psychoactive Substances

S28

Metabolism of Synthetic Cannabinoids THJ-018 and its 5-Fluoro Analog THJ-2201 Following Human Hepatocytes Incubation and High-Resolution Mass Spectrometry

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Background/Introduction: In 2014, THJ-018 and THJ-2201, synthetic cannabinoid indazole analogs of JWH-018 and AM-2201, were detected in herbal novel psychoactive substance blends. The National Forensic Laboratory Information System contains 220 THJ-2201 reports, and the drugs' popularity is documented on drug user forums. Due to numerous adverse events, the DEA listed THJ-2201 as Schedule I in January 2015; THJ-018 and THJ-2201 also were scheduled in Japan in August 2014. Despite increasing prevalence, no human metabolism data are currently available, making documentation of intake from urine specimen analysis and assessment of the drugs' pharmacodynamic, pharmacokinetic and toxicological properties challenging.

Objective: The objective of this study is to characterize THJ-018 and THJ-2201 metabolism in human hepatocytes and to identify optimal analytical targets to document their intake.

Method: THJ-018 and THJ-2201 (10 μmol/L) were incubated in human hepatocytes for 3 h and then analyzed on a TripleTOF 5600+ high-resolution mass spectrometer (Sciex). Data were acquired via TOF (time-of-flight) full scan and information-dependent acquisition (IDA) triggered product ion scans. Mass defect filter (MDF) and dynamic background subtraction (DBS) techniques were utilized in the IDA method. Metabolic stability of THJ-018 and THJ-2201 was evaluated in human liver microsomes (HLM). In addition, *in silico* metabolite predictions were performed with MetaSite (Molecular Discovery) and compared to metabolites identified in human hepatocytes.

Result: *In vitro* intrinsic clearances of THJ-018 and THJ-2201 were intermediate and high, respectively. In 3 h hepatocytes incubation samples, 13 metabolites were detected for THJ-018; the major metabolic pathways were hydroxylation on the pentyl chain and further oxidation or glucuronidation. For THJ-2201, 27 metabolites were observed; oxidative defluorination and subsequent carboxylation or glucuronidation, and glucuronidation of mono-oxidized metabolites dominated. Dihydrodiol formation on the naphthalene moiety was observed for THJ-018 and THJ-2201. Predicted THJ-018 metabolites matched well with metabolites identified in the hepatocyte sample, where the top 5 predicted metabolites were detected. Overall performance of MetaSite was not as accurate for THJ-2201 as THJ-018 because of strong underestimation of oxidative defluorination.

C**onclusion/Discussion:** For the first time, we characterized the human metabolism of THJ-018 and THJ-2201 in human hepatocytes. Based on our study, reasonable markers for identifying THJ-018 intake are 4'-carbonyl-THJ-018, 4'-hydroxy-THJ-018 and pent-1'-enyl-THJ-018. Reliable markers for THJ-2201 are 5'-hydroxy-THJ-018, THJ-018 pentanoic acid, naphthalene hydroxylated THJ-2201, and N-oxide THJ-2201. These data provide a solid foundation for forensic and clinical scientists to identify THJ-018 and THJ-2201 intake, and if identified in authentic toxicology cases, could enable linking observed adverse events to these new synthetic cannabinoids.

This study is supported by the National Institutes of Health, Intramural Research Program, National Institute on Drug Abuse.

Keywords: THJ-018, THJ-2201, Hepatocyte Metabolism, Novel Psychoactive Substance

S29

Flakka, *Gravel*, or "Bath Salts": Understanding the Role of Alpha PVP Blood Concentrations in Forensic Toxicology

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Background/Introduction: Alpha PVP (alpha Pyrrolidinovalerophenone) is one of the novel psychoactive substances (NPS) that has reached a fairly significant level of popularity that cannot be overlooked by the drug monitoring community. Media reports of the use of "flakka" and "gravel", both street names for either pure alpha PVP or an adulterated mix, have reached a fever pitch despite being federally scheduled in February of 2014. Reports of alpha PVP use have included hallucinations, psychosis, agitation, cardiovascular problems, seizures, and death.

Objective: There is very limited toxicological information regarding alpha PVP and it can be difficult for toxicologists to assess the role alpha PVP has in a medicolegal investigation. Currently, there are a few case reports marrying history, side effects, and alpha PVP concentrations in biological specimens. The purpose of this work was to evaluate both DUID and postmortem cases where alpha PVP was reported as a positive finding in blood.

Method: Quantitative results were evaluated over the course of 18 months, from July of 2013 to December of 2014. For a case to be eligible for inclusion in the dataset, alpha PVP had to be reported in blood. In addition, some level of general screening had to be performed; the case either included a general screen for common drugs of abuse or a more comprehensive screen. The cases were categorized as either a police case or a postmortem case; then positive findings were compiled and the data evaluated for trends.

Result: Over the course of 18 months, alpha PVP was quantitatively reported in 79 police cases and 28 postmortem cases. Alpha PVP was the only drug detected in the blood of 31 police cases and in 6 postmortem cases. In many cases, additional compounds were found in conjunction with alpha PVP. It was also common to have multiple classes of drugs found in one case, such as alpha PVP being detected with marijuana and benzodiazepines.

Blood concentrations of alpha PVP varied widely. The average and median alpha PVP blood concentrations in police cases were 64 and 35 ng/mL, ranging from 3.4-440 ng/mL. In cases where only alpha PVP was detected, the average and median concentrations were 88 and 64 ng/mL, ranging 3.8-440 ng/mL. For postmortem cases, average and median alpha PVP blood results were 237 and 100 ng/mL, ranging 3.5-1500 ng/mL.

Conclusion: Alpha PVP is one novel psychoactive substance that started becoming popular in 2012; it has now reached a point where it is a regular occurrence in both postmortem and DUID casework. It is clear from the data listed above that Alpha PVP is a prominent NPS, and the presence of this drug has to be evaluated in context for the entire case.

Keywords: Alpha PVP, Novel Psychoactive Substances, Bath Salts

S30

Three Fatalities Associated with the Synthetic Cannabinoid AB-CHMINACA

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Introduction: In Spring 2014, a new synthetic cannabinoid, N[(1S)-1-(aminocarbonyl)-2-methylpropyl]-1-(cyclohexymethyl)-1H-indazole-3-carboxamide, also known as AB-CHMINACA, emerged in our postmortem toxicology casework. This cannabinoid is in the indazole carboxamide class of synthetic cannabinoids and is structurally distinct from earlier generations of compounds. At the first time of detection in our casework, the substance was not considered a controlled substance by the United States Federal government, but in January 2015, the Drug Enforcement Administration (DEA) placed AB-CHMINACA into Schedule I of the Controlled Substances Act (CSA).

Objective: We present an analytical method for the quantitative detection of AB-CHMINACA in postmortem blood specimens and describe three postmortem cases, to include pertinent autopsy findings and decedent histories, together with the quantitative results for AB-CHMINACA determined in postmortem blood.

Method: Routine screening was completed for opiates/oxycodone and cannabinoids via an enzyme linked immunosorbent assay (ELISA), volatiles by headspace gas chromatography with flame ionization detection (GC-FID), and a comprehensive drug screen by liquid chromatography time of flight mass spectrometry (LC/ToF). Synthetic cannabinoid analysis was performed via liquid-liquid extraction at pH 10.2 into hexane:ethyl acetate and liquid chromatography tandem quadrupole mass spectrometry (LC/MS/MS). The analytical method was validated according to in-house validation standard operating procedures as a quantitative assay. Linearity, accuracy and precision, carryover, exogenous drug interferences, and ion suppression were assessed during method validation.

Result:

Case 1: A 45 year old male was found unresponsive in his bedroom. There were 22 empty packages of herbal incense products found in his room. Pathological findings at autopsy included severe pulmonary edema and congestion (left lung, 1,100 grams and right lung, 1,170 grams). Concentric ventricular hypertrophy was also documented. The postmortem heart blood was positive for AB-CHMINACA (7.2 ng/mL). No other substances were detected. Cause and manner of death was certified as accidental drug overdose by synthetic cannabinoid.

Case 2: A 24 year old female was found unresponsive after being involved in a motor vehicle collision while driving. She was resuscitated at the scene and transported to the hospital. She sustained minor blunt force injuries in the form of abrasions and contusions and a dislocated left hip. She became unresponsive in the hospital and was unable to be resuscitated. Pathological findings at autopsy included bilateral pulmonary edema and congestion along with general visceral congestion. The postmortem heart blood was positive for AB-CHMINACA (3.4 ng/mL) and norfluoxetine (368 ng/mL). The cause and manner of death was determined to be accidental AB-CHMINACA intoxication in the setting of a motor vehicle collision.

Case 3: A 28 year old male was involved in an early morning motor vehicle collision when he hit a telephone pole and struck another vehicle. He was pronounced dead at the scene. A hand-rolled cigar containing a green leafy substance was found in his right hand. At autopsy, considerable trauma, including rib fractures, ruptured pericardium and aorta, and marked bilateral hemopneumothorax was noted. The postmortem heart blood was positive for AB-CHMINACA (10 ng/mL) and alprazolam (64.2 ng/mL). The cause of and manner of death was certified as injuries sustained from motor vehicle accident in the setting of AB-CHMINACA and alprazolam.

Conclusion: AB-CHMINACA was an emerging federally uncontrolled substance in early 2014. The validated analytical method proved to be accurate and reliable for the detection and quantitative analysis of AB-CHMINACA in blood specimens. We detected AB-CHMINACA in three unrelated postmortem cases. With the paucity of available published literature specifically on AB-CHMINACA, we believe these case reports to be a good addition to existing reports surrounding synthetic cannabinoids in postmortem toxicology.

Keywords: Synthetic Cannabinoids, AB-CHMINACA, Fatality

S31

Artifactual Production of Oxcarbazepine from a Carbamazepine Metabolite

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Background: Oxcarbazepine, a 10-keto derivative of carbamazepine, is used clinically as an anticonvulsant. However, oxcarbazepine is not a metabolite of carbamazepine nor is carbamazepine a metabolite of oxcarbazepine. In 2010, Johnson *et. al.* reported the artifactual production of carbamazepine in the GC/MS injector port from a metabolite of oxcarbazepine (10,11-dihydro-10-hydroxycarbamazepine) but there are no reported studies demonstrating the artifactual formation of oxcarbazepine from carbamazepine or its metabolites. This is a brief report of a study demonstrating the formation of oxcarbazepine from the carbamazepine metabolite carbamazepine-10,11-epoxide.

Objective: This report will provide the results of studies performed using pure standards, spiked drug-free urine and real patient samples which demonstrate the formation of oxcarbazepine from carbamazepine-10,11-epoxide.

Method: Drug standards were either diluted in organic solvent containing internal standard and injected without further extraction or spiked into drug-free urine and processed using liquid/liquid extraction. Two urine samples from patients prescribed carbamazepine were also subjected to liquid/liquid extraction. Qualitative analysis was performed using full scan GC/MS analysis on an Agilent 6890/5975. The two patient samples were also subjected to quantitative LC/MS/MS analysis on an Agilent 1200 Series/Triple Quadrapole G6410A with an LOQ of 150 ng/mL. There was no documentation of prescriptions for oxcarbazepine.

Result: Using drug standard solutions, carbamazepine at an on-column concentration of 100 ng did not produce oxcarbazepine whereas carbamazepine-10,11-epoxide at an on-column concentration of 20 ng produced oxcarbazepine when analyzed by GC/MS. Carbamazepine-10,11-epoxide standard spiked into drug-free urine at a concentration of 5000 ng/mL (on-column concentration of 100 ng) and extracted using a liquid/liquid technique produced oxcarbazepine when analyzed by GC/MS. Oxcarbazepine is detectable using qualitative GC/MS analysis at a concentration of 200 ng/mL (on-column concentration of 4 ng) when spiked into drug-free urine and extracted using a liquid/liquid technique. The two patient samples subjected to full scan GC/MS analysis demonstrated oxcarbazepine peaks in addition to carbamazepine and a variety of carbamazepine metabolites including carbamazepine-10,11-epoxide. These two samples did not produce oxcarbazepine when analyzed by LC/MS/MS.

Discussion: These results demonstrate that oxcarbazepine is produced during GC/MS analysis from carbamazepine-10,11-epoxide. When interpreting GC/MS analytical data it is important to understand that drugs may be artifactually produced. The major pathway of carbamazepine biotransformation is via 10,11-epoxide formation with subsequent hydrolysis to 10,11-dihydroxycarbamazepine. Given that carbamazepine-10,11-epoxide is a known urinary metabolite of carbamazepine, the presence of this metabolite in the urine of an individual prescribed carbamazepine would be expected. If the analytical GC/MS data shows carbamazepine or any of its metabolites a cautionary approach should be taken before reporting oxcarbazepine.

Conclusion: The data demonstrates the artifactual production of oxcarbazepine from carbamazepine-10,11-epoxide during GC/MS analysis. Caution must be taken by laboratories when reporting oxcarbazepine to ensure the reported result is a true reflection of drug use.

Keywords: Oxcarbazepine, Carbamazepine, Carbamazepine-10,11-epoxide

S32 ***"NIJ Funded"***

Identification of Major Metabolites in Human Blood and Urine Associated with the Ingestion of Methylone and Dimethylone

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Background/Introduction: Beta-keto-3,4-methylenedioxydimethylamphteramine (bk-MDDMA), commonly referred to as dimethylone, is structurally similar to the more commonly abused methylone. Methylone, the β-keto derivative of MDMA, is one of the compounds often present in drugs distributed under the label "Molly". Although some metabolism data are available on emerging novel psychoactive substances (NPS), the market is constantly changing, and new drugs with unstudied metabolic pathways continue to be introduced and popularized as recreational drugs, it is important to identify their metabolites as markers use and verify their presence in authentic samples from drug users. Methylone, ethylone, and other closely related compounds have been shown to metabolize through pathways that include N-dealkylation, N-hydroxylation, β-keto reducation and demethylenation followed by O-methylation, dimethylone remains

Objective: The purpose of this presentation is to report on results from the analysis of blood, urine and oral fluid samples obtained from volunteers attending an electronic dance music (EDM) festival for the presence of indicators of use of emerging NPS. Blood and urine samples screening positive for methylone and/or dimethylone were further investigated for the presence of candidate metabolites that had been produced *in vitro* using human liver microsome (HLM) incubations of the parent drugs. The metabolites present in the authentic specimens were evaluated to determine if it is possible to distinguish which illicit substance was ingested.

Method: Metabolites of methylone and dimethylone were produced by incubating methylone or dimethylone with pooled human liver microsomes (HLM). Phosphate buffer (pH 7.4) was spiked with 5000 ng of methylone or dimethlyone. NADPH, a co-factor for the enzymatic reaction, was added to the buffer solution and allowed to incubate for 2 hours at 37°C. Following filtration, the samples were analyzed using a Waters Acquity UPLC® I Class coupled to a Waters Xevo® G2-S QTOF to generate exact mass data. Incubated samples were compared with controls that were incubated without the essential NADPH co-factor to help identify products of metabolism.

Human blood samples with paired urine samples from drug using were extracted using a basic liquid-liquid extraction using 0.1 M borate buffer (pH=10.4) into n-butyl chloride and ethyl acetate for an LC-QTOF screen. The organic phase was evaporated to dryness and reconstituted in 90:10 5mM ammonium formate and 0.1% formic acid in acetonitrile and analyzed using LC-QTOF. Human blood and urine samples that had screened positive for methylone or dimethylone by LC-QTOF were further examined using extracted ion chromatograms for the exact masses of candidate metabolites produced using the HLM incubation procedure.

Result: Dimethylone incubations were compared to results from in vitro metabolism of methylone, as dimethylone was seen to metabolize into methylone by N-dealkylation, and then further metabolized by demethylenation of methylone. However, the other products of methylone metabolism (from N-dealkylation and N-hydroxylation) were not observed in the dimethylone incubations. Dimethylone also metabolized by demethylenation into 3,4-dihydroxy-N,N-dimethylcathinone followed by methylation to either 3-hydroxy-4-methoxy-N,N-dimethylcathinone or 4-hydroxy-3-methoxy-N,N-dimethylcathinone which are unique metabolites of dimethylone relative to methylone. Authentic blood and urine samples screening positive were processed against a metabolite library and found to contain several of these metabolites.

Conclusion/Discussion: The combined results from analysis of mass spectrometric data, and HLM incubations, and analysis of urine and blood from authentic cases of methylone or dimethylone ingestion allowed identification of several major metabolites in humans. The metabolic products observed in the methylone incubations resulting from hydroxylation or dealkylation were not seen as products of dimethylone metabolism. The presence of the unique dimethylone metabolites would indicate the ingestion of dimethylone; however, due to the similar metabolic pathway of methylone and dimethylone it cannot be determined if co-ingestion occurred.

Keywords: Dimethylone, Methylone, Metabolism

S33 ***"NIJ Funded"***

Detection of Novel Psychoactive Substances in Blood and Oral Fluid from Attendees at an Electronic Dance Music Festival

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Background/Introduction: High rates of Novel Psychoactive Substances (NPS) and research chemicals have been documented through surveys of Electronic Dance Music (EDM) festival attendees, and reflected in online discussion groups associated with EDM culture. NPS used within the United States has recently been a focus of media attention due to drug-related deaths and mass hospitalizations or medical aid calls. Opportunistic evaluation of recreational drug use within EDM populations allows for the unique opportunity to collect authentic biological samples as an alternative to human dosing experiments, which are typically not considered safe to administer in a controlled setting for drugs with an unknown toxic effect profiles. Oral fluid is of increasing interest as an alternative biological specimen for drug detection. We have previously reported methods for detecting NPS in blood and urine, and several methods for detecting common drugs of abuse have been developed for oral fluid, including amphetamine, cannabis, cocaine, opiates, and benzodiazepines. However, there are few evaluations of the detectability of NPS in oral fluid or their relative concentrations in blood and oral fluid.

Objective: The purpose of this investigation was to compare oral fluid samples with paired blood specimens collected from subjects admitting to NPS drug use to determine the extent to which the two specimens can be correlated in terms of rates of drug detection and whole blood to oral fluid ratios.

Method: Participants were recruited during an EDM festival in Florida during 2014 and 2015 and asked to provide a blood, urine, and oral fluid samples for comprehensive drug testing. Three hundred and eighty-four oral fluid specimens were collected over the two years. A total of 126 oral fluid samples also had a paired blood specimen. Subjects also completed a survey about their drug use. Oral fluid and blood samples were screened using liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF). Six NPS (methylone, dimethylone, butylone, ethylone, 4-FA, alpha-PVP) were confirmed by liquid chromatography tandem mass spectrometry (LC-MS/MS) with a detection limit of 5 ng/mL in blood and 20 ng/mL in oral fluid. Confirmatory methods for blood and oral fluid were validated according to SWGTOX guidelines.

Result: Of the 384 oral fluid samples collected over two years, 94 were positive for NPS including alpha-PVP, dimethylone, methylone, butylone, ethylone, and MDMA. Additional analytes of interest, including heroin, ketamine and hordenine were also detected in the oral fluid. Of the oral fluid samples in this population that were positive for any drug, approximately half were positive for one or more NPS (53%). In comparing the results of blood and oral fluid samples, there was good agreement between the two matrices for the detection of common drugs of abuse, therapeutic compounds, and NPS. There were however seven instances where NPS drugs (alpha-PVP, dimethylone, and ethylone) were detected in the oral fluid, but not detected in the blood specimens, but only two subjects testing positive in blood for an NPS that were not found in the oral fluid, at the cut-offs selected.

Conclusion/Discussion: The EDM festival culture has been largely understudied in the United States. However, this population has proved to be an invaluable resource in terms of learning about patterns of recreational drug use and emerging NPS. The data collected demonstrate the utility of oral fluid as a specimen for detecting NPS use. The ease of collection of oral fluid makes it a useful specimen for collecting data about NPS drug trends at these events. This data can be useful for educating users about risks associated NPS, provide opportunities for harm reduction, and enable forensic laboratories to target innovative testing strategies for impairment or death investigations associated with these events.

Keywords: Novel Psychoactive Substances, Oral Fluid, Blood

S34

Definitive Multi-Analyte Drug Testing in Urine by UPLC-MS/MS: An Alternative to Presumptive Screening by Immunoassay

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Introduction: Drug screening is an essential analytical tool for detection of therapeutic, illicit and emerging drug use. While postmortem drug identification relies heavily on limit of detection screening using definitive methods (GC-MS and LC-MS technology), drug screening in the court, employment and clinical settings is generally performed by immunoassays based on administrative threshold criteria. Replacement of immunoassays with multi-analyte definitive methods of screening has been hampered by complex sample preparation, long chromatography time and ion-source matrix effects.

Objective: The aim of this study was to develop and validate a rapid and threshold-accurate urine multi-analyte screen for 31 drugs and/or their metabolites including benzodiazepines, opiates, opioids, cocaine, phencyclidine, amphetamines and cathinones. A novel threshold analyte calibration (TAC) technique was used to achieve threshold-accurate drug detection with UPLC-MS/MS analysis.

Method: A simple preparation of urine (200µL), involving rapid glucuronidase hydrolysis followed by dilution and filtration, was performed in 96-well-plate format followed by direct UPLC-MS/MS analysis of the filtrate. Dual transition-ion monitoring for the detection of drugs and metabolites was optimized in a three minute MS/MS acquisition program following chromatographic separation on an ACQUITY UPLC BEH phenyl column (1.7 um, 2.1 x 50mm). TAC technique involved testing urine with (spiked) and without (neat) reference-analytes spiked at the administrative threshold concentration. The analyte specific thresholds used in the assay ranged from 10 to100 ng/mL. A recovery standard (methapyrilene) was added to neat and spiked samples to verify precision of injection volume. TAC ratio of neat to spiked ion area was determined for each analyzed specimen and was calibrated for each analyte using calibrator urine containing threshold concentration of the analytes.

Result: Validation of accuracy and precision (%of target, %CV) at above and below threshold concentrations was determined by replicate analysis of control urine pools containing 50 (96,8), 75 (99,9), 125 (100,9) and 150 (100,9) percent of threshold concentration. Range of accuracy (91-116% of target) and precision (2-15%) was within 20% for all analytes. Within run coefficient of variation of TAC ratio for calibrators prepared in seven urine pools was within 15% (mean 6.4%; range 3.2-13.6%). Matrix effect studies demonstrated a mean absolute recovery of 83% (range 43-113%) across all analytes, based on transition ion area of analyte-supplemented urine compared to supplemented mobile phase. Within-analyte matrix effect varied significantly between urine specimens and was directly correlated with urine creatinine concentration for earlier eluting analytes. TAC ratio normalized the variable matrix effect on ion recovery and allowed consistent and threshold-accurate detection of analytes. Full concordance with proficiency testing results and with definitive confirmation testing results for de-identified case specimens further demonstrated the accuracy and selectivity of drug and metabolite detection by the definitive method of screening.

Conclusion: TAC technique coupled with rapid UPLC-MS/MS analysis offer the potential for definitive drug screening as an alternative to presumptive immunoassay screening for both forensic and clinical applications. The TAC technique of LC-MS/MS screening is also applicable to threshold-accurate detection of rapidly emerging designer drugs where deuterated internal standard are not yet available.

Keywords: Drug Screening, LC-MS/MS, Immunoassay Alternative

S35

Headspace Gas Chromatography with Simultaneous Flame Ionization and Mass Spectrometric Detection: An Approach to the Analysis of Forensic Alcohol Samples

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Introduction: For years the field of forensic alcohol toxicology has relied heavily upon gas chromatography with flame ionization detector (FID) as the primary means of analysis. Currently, the most popular approach utilizes gas chromatographs equipped with dual columns and dual FID detectors. This type of analysis yields data that provide dissimilar elution orders for reported analytes and increases the specificity of analysis. The technique to be discussed here, couples a single FID detector with the structural specificity of a mass spectral detector (MSD). The data produced by this single column analysis, yields a FID chromatogram used for quantitation as well as a mass spectral chromatogram for structural identification. Both of these chromatograms share elution order as well as retention times, thus simplifying the creation of a final report document and quantitation upload into a database.

Objective: The overall goal was to modify an existing methodology to provide a method that includes fully integrated reporting processes and easy result upload into a database. This is necessitated by an annual volume of approximately twenty thousand forensic alcohol cases.

Method: An Agilent 7890B Gas Chromatograph equipped with both FID and an Agilent 5977 MSD was coupled with an Agilent 7697A Headspace Sampler to develop a rapid and robust method to effectively quantitate and structurally identify ethanol, methanol, acetone, and isopropyl alcohol. The FID signal was utilized for the quantitative results. Instrument control and data processing was performed using Agilent MassHunter version 7.02. Chromatographic separation was achieved using an Agilent DB-ALC1 thirty meter column.

Result: The separation and quantitation of ethanol, methanol, acetone, and isopropyl alcohol were all achieved with a linearity (r^2>0.99) and detection limits of 0.015 g/dL. The final report document was created and utilized a single chromatogram to represent both detectors. Results for the quantitation, the mass spectrum for each analyte, and a library search result were integrated into the final report document. This document was then used to import the results into a database for insertion into the laboratory's final report for a particular case.

Conclusion: The analysis and reporting method provides a robust and complete procedure for the quantitation of ethanol, methanol, acetone, and isopropyl alcohol by FID, while simultaneously providing mass spectral confirmation. The final report document is easily customizable and the data is effortlessly imported into a database for simplified and uniform reporting. Other volatile substance analysis is possible with this type of technique, and will be investigated in the future.

Keywords: Blood Alcohol, MassHunter, HS-GC-FID-MS

S36

Simultaneous, Direct Quantification of Plasma Buprenorphine, Naloxone and Phase I and II Metabolites by LC-MS/MS

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Introduction: In the United States, methadone is the only medication-assisted opioid addiction treatment approved for breastfeeding women. Buprenorphine (BUP) monotherapy was successfully implemented as a treatment strategy for opioid-addicted pregnant women but its safety during breastfeeding has not yet been fully evaluated. Combination BUP and naloxone (NAL) therapy, known as Suboxone®, is successfully utilized for opioid addiction treatment, but there is concern for naloxone's potentially adverse effects on the fetus during *in utero* exposure.

Objective: To develop a comprehensive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for simultaneous quantification of plasma BUP, NAL and phase I and II metabolites to study BUP and NAL trans-placental distribution during maternal sublingual Suboxone treatment.

Method: Blood was collected into sodium heparinized tubes, spun into plasma, and frozen until analysis. Plasma (100µL) was diluted with 2mL 0.1M phosphoric acid and centrifuged prior to loading onto preconditioned strong cation-exchange polymeric solid phase extraction (SPE) columns (Strata X-C, 60mg/3mL). Analytes were eluted with 3mL dichloromethane:isopropanol: ammonium hydroxide (70:26:4, v/v/v), dried completely under nitrogen at 35°C and reconstituted in 125µL mobile phase (85:15, A:B, v/v). Extracted specimens were placed into a 4°C refrigerated autosampler and 50µL was injected onto a Shimadzu Prominence UFLCxr coupled to a SCIEX 5500 QTRAP MS for analysis. Analytes were separated via gradient elution at 0.5mL/min with 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B) on a Restek Raptor Biphenyl column (100x2.1mm, 2.7µm) at 40°C. Data were acquired via multiple reaction monitoring (MRM) with positive electrospray ionization (ESI); two MRMs were acquired for each analyte. The method was fully validated according to SWGTOX guidelines and was applied to quantify BUP, norbuprenorphine (NBUP), BUP-glucuronide (BUP-Gluc), NBUP-Gluc, NAL, nornaloxone (NNAL), and naloxone-N-oxide (NAL-N-Ox) in maternal and umbilical cord plasma collected from 3 women treated with sublingual Suboxone during pregnancy.

Result: Limits of quantification (LOQ) were 0.025-0.25µg/L with linearity up to 50µg/L (except 25µg/L for NAL and BUP-Gluc). A $1/x^2$ weighting factor was employed for calibration curves to compensate for heteroscedasticity. Extraction efficiencies and matrix effects were 69-99% and 25-67% suppression, respectively. Matched deuterated internal standards, whenever available, compensated for matrix effects. Intra-batch imprecision was ≤10.6% (% relative standard deviation, RSD), inter-batch imprecision was ≤12.6%RSD and bias was within ±13% of target for all analytes. Analytes were stable (≤18.4% difference) when stored at 24h at room temperature, 72h at 4°C, and after three freeze/thaw cycles. No carryover was observed in negative specimens after injecting samples at twice the upper LOQ. With the exception of NAL-N-Ox, all analytes were quantified in paired maternal and umbilical cord plasma specimens from three mothers. Maternal median (range) concentrations were 0.1µg/L (<LOQ-0.1µg/L) NAL, 0.4µg/L (0.2-0.4µg/L) NNAL, 0.9µg/L (0.4-1.0µg/L) BUP, 0.6µg/L (0.6-0.7µg/L) NBUP, 2.6µg/L (1.5-4.2 µg/L) BUP-Gluc, and 11.8 µg/L (11.4-15.9 µg/L) NBUP-Gluc. Umbilical cord median (range) concentrations were 0.1µg/L (0.1-0.2µg/L) NAL, 0.5µg/L (0.2-0.5µg/L) NNAL, 0.5µg/L (0.1-0.7µg/L) BUP, 0.3µg/L (0.3-0.4µg/L) NBUP, 1.4µg/L (0.9-3.4µg/L) BUP-Gluc, and 16.6µg/L (9.5-18.8µg/L) NBUP-Gluc.

Conclusion: This highly sensitive LC-MS/MS method was fully validated according to SWGTOX guidelines for quantifying BUP, NAL and metabolites in only 100µL plasma. The method was applied to paired maternal and umbilical cord plasma clinical specimens to examine BUP and NAL maternal-fetal transfer.

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Keywords: Buprenorphine, Naloxone, Plasma

S37
SAMHSA's Role in Establishing Drug Testing Standards for Contemporary Drugs

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Introduction: The Substance Abuse and Mental Health Services Administration (SAMHSA) establishes goals and objectives for the administration of a national program designed to promote a drug free federal workforce. SAMHSA sets technical standards and provides assistance in the implementation of Mandatory Guidelines for urine drug testing programs. Another goal of SAMHSA is to consider use of new technologies for improvements in the accuracy and validity of drug tests over existing methodologies. Considerations are given to alternate matrices where there is sufficient scientific support for inclusion in the federal workplace drug testing program.

Objective: SAMHSA's goal of supporting existing technologies and development of new technologies for drug testing led to design of a series of studies of contemporary drugs. The proliferation of high potency cannabis products could increase the risks of secondhand exposure to cannabis smoke. Ingestion of edible cannabis products could result in altered patterns of disposition. The purpose of the current studies was to evaluate use of high potency cannabis by different routes of administration.

Method: Drug dosing studies require the highest level of oversight to ensure the safety of participants and scientific validity of the resulting information. Conduction of clinical studies required multiple efforts: development of complete protocols; close collaboration with clinical scientists; protocol approvals from Institutional Review Boards; submitting Investigational New Drug Applications for approval to conduct studies from the Food and Drug Administration; approval from the Drug Enforcement Agency for obtaining and controlling needed supplies of cannabis; obtaining cannabis supplies from the National Institute on Drug Abuse; analysis of cannabis potency; financial support for conducting studies; oversight of clinical studies by RTI International; shipment of biological specimens to commercial laboratories for analyses; data review; and preparation of data for public dissemination.

Result: Two key clinical studies were completed with high potency cannabis. The first study evaluated the effects of extreme secondhand cannabis smoke exposure in non-smokers who were in a room with individuals actively smoking cannabis. The study evaluated exposure from two potencies of smoked cannabis and the effects of room ventilation. Analysis of urine specimens by immunoassay (IA) and confirmation tests indicated that subjects inhaled sufficient tetrahydrocannabinol to produce multiple positive result at thresholds used in the private sector for 2-22 hours (but only one positive with one was produced at DHHS thresholds) and up to 3 hours for oral fluid. The second study evaluated oral ingestion of three potencies of cannabis brownies. Analysis of urine and oral fluid specimens indicated THC metabolites were excreted in urine for up to 216 hours; THC was detectable in oral fluid for up to 22 hours and THCCOOH for up to 94 hours.

Conclusion: SAMHSA has taken a lead role in the evaluation of high potency cannabis by conducting key studies in human volunteers. The results of these studies provided a large array of important pharmacological information (e.g., behavior, physiology, and performance) and toxicological information on the disposition of cannabinoids in urine, oral fluid, blood and hair specimens. These data will contribute important information on the scientific validity of drug testing.

Keywords: Cannabis, SAMHSA, Mission, Research

S38

The Pharmacodynamic Effects of Passive Exposure to Secondhand Cannabis Smoke

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Introduction: The increasing use of highly potent strains of cannabis prompted this new evaluation of human exposure to secondhand cannabis smoke. The study was designed to produce extreme cannabis smoke exposure conditions tolerable to drug-free non-smokers.

Objective: Evaluate whether severe secondhand cannabis smoke exposure was associated with subjective feelings of intoxication, cardiovascular changes, or impacted cognitive performance.

Method: Secondhand cannabis smoke exposure sessions were conducted. Each exposure session lasted for 60 minutes and involved 6 drug-free individuals seated in a Plexiglass chamber alternately with 6 experienced cannabis smokers while they consumed cannabis *ad-libitum*. In Session 1 the smokers consumed cannabis containing 5.3% THC in an unventilated environment; Session 2 involved consumption of cannabis containing 11.3% THC in an unventilated environment, and Session 3 involved consumption of cannabis containing 11.3% THC in a ventilated environment. At baseline and nominal time points post-exposure, smokers and non-smokers rated subjective drug effects on a 15-item visual analog scale (VAS); had vital signs measured, and completed a battery of cognitive performance assessments that measured psychomotor function (Digit Symbol Substitution Task; DSST), working memory (Paced Auditory Serial Addition Task; PASAT), and divided attention.

Result: Exposure to secondhand cannabis smoke in Session 2 (11.3% THC, unventilated) produced detectable cannabinoid concentrations in blood (THC range = 1.2-5.6 ng/mL; THC-COOH range = 0-5.1 ng/mL) and urine (THC-COOH range 5.5 – 57.5 ng/mL), minor increases in heart rate, mild to moderate self-reported sedative drug effects, and impaired performance on the DSST among non-smokers. Exposure under ventilated conditions resulted in much lower blood cannabinoid concentrations and did not produce sedative drug effects, impairments in performance, or positive urine screen results. Exposure to secondhand smoke from cannabis containing 5.3% THC produced intermediate effects, but interpretation of potency is limited because participants smoked less low potency cannabis compared with high potency cannabis. Smokers exhibited expected reports of subjective intoxication. Cardiovascular effects were generally mild and cognitive performance effects were not observed among smokers.

Conclusion: Short-term exposure to high-intensity smoke from combusted cannabis can produce subjective intoxication, mild cognitive impairment and cardiovascular effects. These effects were consistent with, but lower in severity compared with those observed among active smokers. There was a significant effect of room ventilation on all outcomes. Evaluation of potency was limited due to variability in the amount of cannabis consumed by smokers across sessions. Pharmacodynamic effects varied systematically relative to observed blood THC concentrations.

Keywords: Cannabis, Secondhand Smoke, Tetrahydrocannabinol

S39

The Pharmacodynamic Dose Effects of Oral Cannabis Administration

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Introduction: Medical and non-medical use of cannabis is increasing and now includes legal distribution of cannabis products in many states. This has led to a proliferation of orally administered "edible" cannabis products. Few controlled studies have been conducted evaluating the pharmacodynamic effects of oral cannabis and there is controversy regarding what constitutes an appropriate dose for retail sales.

Objective: Evaluate the dose effects of orally administered cannabis on subjective ratings of intoxication, objectively measured heart rate and blood pressure, and cognitive performance on tasks of psychomotor ability, divided attention, and working memory.

Method: Non-drug users were recruited to complete a single oral cannabis administration session. A total of 18 participants completed the study; 6 participants (3M, 3F) were administered one of three doses: 100 mg, 250 mg, or 500 mg of dried cannabis plant material containing roughly 10 THC% (yield of approximately 10, 25, and 50 mg THC per dose). Cannabis for this study was obtained from the NIDA Drug Supply Program. Cannabis was pre-heated for 30 minutes at 325 degrees to facilitate conversion of the acid form of THC (THC-A) to free THC. This process of THC decarboxylation is required to facilitate the transfer of THC across the blood brain barrier. The cannabis was then baked into brownies using a commercial brownie mix. At baseline and nominal time points up to 8 hours post-exposure, participants rated subjective drug effects on a 15-item visual analog scale (VAS); had vital signs measured, and completed a battery of cognitive performance assessments that measured psychomotor function, working memory, and divided attention (Digit Symbol Substitution Task (DSST), Paced Auditory Serial Addition Task (PASAT), and a divided attention task). Blood, urine, and oral fluid samples were obtained before and post drug administration.

Result: Subjective ratings of "drug effect", heart rate, psychomotor ability (DSST number correct) and working memory (PASAT total correct) were qualitatively dose dependent. There was little difference between the 250 and 500 mg cannabis doses, but both indicated greater subjective intoxication and worse psychomotor performance compared with the 100 mg dose. Ratings of "good drug effect" did not differ by dose, but ratings of "unpleasant drug effect" were higher following the 250 mg and 500 mg doses compared with the 100 mg dose. Peak drug effects occurred 1.5 – 3 hours post exposure and effects typically lasted for 6-8 hours. Mean peak increases in heart rate ranged from 13 to 24 bpm and occurred 1 hour after drug administration. No changes in blood pressure were observed. One female experienced an intense period of anxiety following the 250 mg dose and 2 females vomited 3 hours after consuming the 500 mg dose. Blood and oral fluid levels of cannabinoids were low for all participants, and undetectable for 2 participants following administration of the 100 mg dose.

Conclusion: Oral administration of cannabis resulted in dose-dependent effects on subjective ratings of total and unpleasant drug effect, measured heart rate, psychomotor ability, and working memory performance. At the 250 mg and 500 mg cannabis doses, adverse events were reported and observed, which seemed to drive the increase in total drug effect relative to the 100 mg dose. Decrements in cognitive performance consistent with the severity of self-reported intoxication were observed. However, the duration and magnitude of self-reported intoxication and performance impairment did not correspond with quantitative levels of THC, 11-OH-THC, and THC-COOH in blood or oral fluid, which were uniformly low. In fact, concentrations of all analytes were <5ng/mL, the most commonly used cut-off for roadside drug testing. This study was limited by a lack of placebo condition, but a second phase of the study that includes placebo and a full dose crossover within participants is under way to address that limitation.

Keywords: Cannabis, Tetrahydrocannabinol (THC), Edibles, Oral Administration

S40
Oral Administration of Cannabis in Brownies

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Introduction: As US state-sanctioned legalized cannabis programs expand, a concomitant proliferation of the use of "edible" (orally administered) cannabis products has increased. The absorption and distribution of tetrahydrocannabinol (THC) and metabolites in different biological matrices following ingestion of edible cannabis products has not been well documented.

Objective: Perform a comprehensive evaluation of the disposition of THC and metabolites in urine, oral fluid, and blood following ingestion of cannabis-containing brownies.

Method: Single dosing sessions were conducted in which six unique drug-free individuals consumed a chocolate brownie containing 100 mg, 250 mg, or 500 mg of cannabis. THC content was approximately 10%, yielding brownies with approximately 10, 25, or 50 mg THC. Cannabis was pre-heated for 30 minutes at 325º F to facilitate decarboxylation of THC precursors. Brownies were then prepared using a commercial brownie mix in accordance with the manufacturer's instructions, and the cannabis was stirred into the brownie batter before baking. Specimens were collected at baseline and at timed intervals through 216 h following dosing. One brownie containing 500 mg of marijuana was analyzed to confirm the validity of the preparation method. All specimens were analyzed by GC/MS (urine) or LC/MS/MS (oral fluid and blood). The respective LOQ concentrations (ng/mL) were: urine, THC carboxylic acid (THCCOOH), 0.75; oral fluid, THC 1, THCCOOH, 0.02; and blood, THC, 0.5, 11-hydroxy-THC (11-OH-THC), 0.5, and THCCOOH, 0.5. Urine and oral fluid specimens were also analyzed by immunoassay.

Result: THC contained in the 500 mg Marijuana brownie was 49.7 mg. Mean (range) THCCOOH Cmax urine concentrations (ng/mL) were: 100 mg cannabis, 106.8 (33.8 - 277.9); 250 mg cannabis, 335.1 (74.8 - 728.6); and 500 mg cannabis, 712.6 (215.5 - 1024.8). Corresponding Tmax (h) were: 9.7 (4.0 - 22.0); 6.7 (4.0 - 8.0); and 11.2 (3.0 - 22.0). Urine detection times (last positive specimen >LOQ) ranged from 74-216 h across doses. Mean (range) THC Cmax oral fluid concentrations (ng/mL) were: 100 mg cannabis, 191.5 (47.0 - 412.0); 250 mg cannabis, 477.5 (70.0 - 1128.0); 500 mg cannabis, 597.5 (350.0 - 1010.0). Corresponding Tmax (h) were: 0.2 (0.2 - 0.5) for all doses. THC detection times ranged from 1.5-22 h. Mean (range) THCCOOH Cmax oral fluid concentrations (pg/mL) were: 100 mg cannabis, 50.8 (0.0 - 231.0); 250 mg cannabis, 139.7 (23.0 - 251.0); and 500 mg cannabis, 314.3 (0.0 - 822.0). Corresponding Tmax (h) were: 1.0 (0.0 - 3.0); 9.8 (3.0 - 30.0); and 17.4 (0.0 - 54.0). THCCOOH detection times ranged from 0-126 h. Mean (range) Cmax blood concentrations (ng/mL) respectively were: 100 mg cannabis, 1.0 (0.0 - 3.0) THC, 1.0 (0.0 - 2.0) 11-OH-THC, 7.2 (5.0 - 14.0) THCCOOH; 250 mg cannabis, 3.5 (3.0 - 4.0) THC, 3.3 (2.0 - 5.0) 11-OH-THC, 21.3 (12.0 - 39.0) THCCOOH; and 500 mg cannabis, 3.3 (1.0 - 5.0) THC, 3.2 (2.0 - 4.0) 11-OH-THC, 29.3 (16.0 - 44.0) THCCOOH. Detection times for THC, 11-OH-THC, and THCCOOH were in the range of 0-22, 0-12, and 3-94 h, respectively.

Conclusion: Although substantial concentrations of THCCOOH were excreted in urine following oral dosing of cannabis, blood concentrations of THC and metabolites were extremely low; oral fluid concentrations were initially high, but disappeared within a few hours. Thus, oral ingestion of cannabis presented a different pattern of disposition than observed for smoked cannabis. It appears that use of blood tests for evidence of impairment following oral cannabis consumption will be problematic.

Keywords: Cannabis, Oral, Tetrahydrocannabinol, Dose

S41

Passive Exposure to Secondhand Cannabis Smoke. 1) Disposition in Urine, Oral Fluid and Blood

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Introduction: The increasing use of highly potent strains of cannabis prompted this new evaluation of human exposure to secondhand cannabis smoke. The study was designed to produce extreme cannabis smoke exposure conditions tolerable to drug-free non-smokers.

Objective: Perform a comprehensive evaluation of the disposition of tetrahydrocannabinol (THC) and metabolites in urine, oral fluid, and blood of non-smokers following exposure to cannabis smoke.

Method: Three secondhand cannabis exposure sessions were conducted in a Plexiglass chamber. Each session involved 6 drug-free smokers seated alternately with 6 experienced cannabis smokers. Session 1 involved exposure to smoked cannabis containing 5.3% THC in an unventilated environment; Session 2 involved exposure to smoked cannabis containing 11.3% THC in an unventilated environment, and Session 3 involved exposure to smoked cannabis containing 11.3% THC in a ventilated environment. Pre-session and timed collections after exposure were conducted of urine, oral fluid, and whole blood. Smokers were discharged after 8 h; non-smokers remained in the study for 34 h. Urine and oral fluid were screened by immunoassay; all specimens were analyzed by GC/MS (urine) or LC/MS/MS (oral fluid and blood). The respective MS LOQ concentrations (ng/mL) were: urine THCCOOH = 0.75; oral fluid THC = 1; oral fluid THCCOOH = 0.02; blood THC = 0.5; and blood THCCOOH = 0.5.

Result: THCCOOH was detectable (>LOQ) in urine of all non-smokers by GC/MS within 0.25 to 3 h post-exposure. A total of 27 specimens (3 in Session 1, 22 in Session 2, and 2 in Session 3) had THCCOOH concentrations ≥ 15 ng/mL Urine Cmax concentrations (range) of THCCOOH were: Session 1 = 11.2 (1.9-20.1) ng/mL; Session 2 = 28.3 (5.5-57.5) ng/mL; and Session 3 = 7.5 (1.3-15.5) ng/mL. A single screen/confirm (50/15 ng/mL) positive urine specimen was identified by IA/MS (1 of 5 IA panels tested; Session 2; 4 h post-exposure) and multiple positives were obtained at lower cutoff concentrations. Oral fluid Cmax concentrations (range) of THC by MS were: Session 1 = 34.0 (4.9-86) ng/mL; Session 2 = 81.5 (12-308) ng/mL; and Session 3 = 16.9 (1.7-75) ng/mL. Thereafter, THC concentrations dropped rapidly over the next 1-3 h. Oral fluid specimens for non-smokers in the two sessions conducted without ventilation tested positive by immunoassay (4 ng/mL cutoff concentrations) and were confirmed for THC by LCMSMS (2 ng/mL) for up to three hours following cessation of exposure. Maximum THC and THCCOOH concentrations (Cmax) by LC/MS/MS in oral fluid generally occurred in the first collected specimen (Tmax = 0.25 h). Mean (range) Cmax concentrations of THC and THCCOOH in blood were: Session 1 = THC, 1.4 (0.6-1.8) ng/mL, THCCOOH = 1.2 (0.8-1.7) ng/mL; Session 2 THC = 3.1 (1.2-5.6) ng/mL, THCOOH = 2.5 (0-5.1) ng/mL; and Session 3 THC = 0.5 (0-0.9) ng/mL, THCCOOH = 0.2 (0-0.7) ng/mL.

Conclusion: Short-term exposure to high-intensity smoke from combusted cannabis resulted in non-smoker inhalation of sufficient amounts of THC to produce positive tests in all biological matrices at lower cutoff concentrations, but not generally at higher initial test cutoff concentrations in general use by DHHS in Federal workplace drug testing programs.

Keywords: Cannabis, Passive, Tetrahydrocannabinol, Dose

S42

Passive Exposure to Secondhand Cannabis Smoke. 2) Dose, Response, and Implications

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Introduction: The characteristics of drug exposure and the spectrum of effects are described as a dose-response relationship, a fundamental consideration in pharmacology and toxicology. Breathing secondhand cannabis smoke can be considered as a means of drug administration.

Objective: Evaluate the amount (dose) of tetrahydrocannabinol (THC) delivered to non-smokers and potential implications for individuals involved in safety-sensitive activities.

Method: Two secondhand cannabis exposure sessions conducted in this study were designed to evaluate the effect of potency of THC in smoked cannabis (Session 1, 5.3% THC and Session 2, 11.3% THC) on non-smokers in a controlled environmental chamber without ventilation. Session 3 was a repeat of Study 2, but was conducted to evaluate the effect of ventilation on exposure. Following each session, oral fluid and whole blood specimens were collected from non-smokers and smokers at nominally paired times and urine was collected at scheduled intervals. All specimens were analyzed by LC/MS/MS for THC and/or THCCOOH. Paired oral fluid and whole blood THC and THCCOOH concentrations determined by LC/MS/MS were compared to evaluate the degree of linear dependence between the two matrices using linear regression methods. Doses of THC delivered by secondhand smoke were determined by area-under-the-curve (AUC) calculations of THC concentrations in oral fluid and blood. The cumulative total amount of THCCOOH excreted by each non-smoker was compared to 0.1974 mg of THCCOOH; the amount of THCCOOH reported to be excreted in urine over a 7 day period by smokers who smoked a single 3.55% THC cigarette (1).

Result: A composite correlation of all non-smoker participants (n=44) individually paired specimens for THC in oral fluid versus THC in blood yielded a Pearson's product-moment correlation coefficient (r) equal to 0.385 (df=42); significance of the correlation (non-directional) was $p<0.01$. Individual correlations for the six smokers of THC between oral fluid and blood in each session also were generally significant ($p<0.01$), but did not significantly correlate between the two matrices for THCCOOH. AUC calculations of the dose of THC inhaled by non-smokers (NS) relative to smokers (S) based on oral fluid and blood, respectively, were (%NS/S): Session 1 = 4.7%, 2.4%; Session 2 = 11.2%, 5.9%; and Session 3 = 1.4%, 0.3%. The relative amounts of total THCCOOH excreted in urine were (%NS/S): Session 1 = 3.1%; Session 2 = 17.9%; and Session 3 = 3.8%.

Conclusion: Significant correlations of THC in oral fluid to blood for non-smokers suggested that absorption took place rapidly. Further, blood THC underwent rapid distribution to tissues in a similar pattern as observed in smokers. Mean estimates of the THC dose delivered to non-smokers in the most extreme condition (Session 2) ranged across matrices from 4% to 18% relative to smokers. Thus, in the most extreme exposure condition, the effects of passive exposure mimicked, to a lesser extent, active smoking effects. This combined body of data suggest that environmental exposure to cannabis smoke should be avoided by non-smokers and potentially has implications for those who undergo drug testing and those engaged in safety-sensitive activities.

1. Huestis,M.A., Mitchell,J.M., and Cone,E.J. (1996), Urinary excretion profiles of 11-nor-9-carboxy-D^9-tetrahydrocannabinol in humans after single smoked doses of marijuana. *J.Anal.Toxicol.*, **20,** 441-452.

Keywords: Cannabis, Passive, Tetrahydrocannabinol

S43

Quantification of Eleven Cannabinoids and Metabolites in Human Urine by Liquid Chromatography Tandem Mass Spectrometry

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Introduction: Cannabinoids are the most commonly abused illicit drug. Excretion of Δ^9-tetrahydracannabinol (THC), 11-hydroxy-THC (11-OH-THC) and 11-nor-9-carboxy-THC (THCCOOH) is prolonged in chronic frequent cannabis users for up to 3 weeks after abstinence confounding recent intake distinction. We are conducting clinical studies to evaluate whether the THC precursor, Δ^9-tetrahydracannabinolic acid (THCAA), the minor plant cannabinoids cannabigerol (CBG), Δ^9-tetrahydrocannabivarin (THCV) and its metabolite 11-nor-9-carboxy-THCV (THCVCOOH) assist identifying recent intake. A comprehensive method for cannabinoid detection is crucial for proper urine results interpretation and for supporting our clinical research.

Objective: To develop and validate a quantitative liquid chromatography tandem mass spectrometry (LC-MS/MS) method for: THC, 11-OH-THC, THCCOOH, cannabidiol (CBD), cannabinol (CBN), CBG, THCV, THCVCOOH, THCAA, THCCOOH-glucuronide and THC-glucuronide in human urine.

Method: 200 µL urine was fortified with internal standards and clarified via centrifugation at 15,000g, 4°C for 5 min after addition of 500 µL acetonitrile. 550 µL supernatant was transferred to a clean microcentrifuge tube containing 200 µL 5% aqueous formic acid before aspiration 4 times through WAX-S tips (DPX Labs, Columbia, SC, 1 mL tip containing 20 mg resin and 40 mg salt,). 90 µL of upper, organic layer was transferred to a clean microcentrifuge tube containing 210 µL mobile phase A before centrifugation at 15,000g, 4°C for 5 min and transfer to an autosampler vial. Chromatographic separation was achieved using a Kinetex 2.1 x 50 mm, 2.6 µm column (Phenomenex Inc., Torrance, CA) at 40°C on a Nexera LC-30 ultra-high pressure liquid chromatograph (Shimadzu Scientific, Columbia, MD). Gradient elution was performed with mobile phase A) 10 mM ammonium acetate in water and B) 15 % methanol in acetonitrile at 0.5 mL/min; total run time was 14 min. MS/MS data were acquired with a Shimadzu LCMS-8050 triple quadrupole mass spectrometer via electrospray ionization scheduled multiple reaction monitoring (MRM), acquiring data in both positive and negative mode; two MRMs were acquired for all analytes.

Result: Extraction efficiency and matrix effect (N=10) were 34-79% and -10 to 23%, respectively. Linear ranges were 0.5-100 µg/L for THC and THCCOOH, 0.5-50 µg/L for 11-OH-THC, CBD, CBN, and THC-glucuronide, 1-100 µg/L for CBG, THCV, and THCVCOOH, and 5-500 µg/L for THCCOOH-glucuronide ($r^2 > 0.995$). Inter-day analytical recoveries (bias) and imprecision (N=25) were 93.5-111.9 % and 4.4-12.9 % coefficient of variation, respectively. Analyte stability was acceptable (±20 %) at both low and high concentrations (N=3) after 72 h at 4°C and three freeze-thaw cycles. 11-OH-THC, THCCOOH, THCVCOOH, THC-glucuronide and THCCOOH-glucuronide were stable; however, THC, CBD, CBN, CBG, THCV and THCAA decreased 37.3-68.3 % after 21 h at room temperature.

Conclusion: We present a simple, novel and fully-validated urinary quantification method for eleven cannabinoids using an efficient and rapid disposable pipette tip extraction procedure while decreasing solvent consumption. The method has successfully been applied to quantify cannabinoids in urine after cannabis administration.

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

Keywords: LC-MS/MS, Urine, Cannabinoids

S44

Fentanyl and Heroin Cases on the Rise: A Geographical Study of Fentanyl Deaths with Confirmed Exposure to Heroin in North Carolina

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Introduction: The North Carolina Office of the Chief Medical Examiner has seen a 300% increase in heroin deaths in the past six years. Near the end of 2013, cases positive for probable non-pharmaceutical grade fentanyl and 6-acetylmorphine (6-AM)/morphine started appearing. The question of whether heroin was being laced with fentanyl required investigation.

Objective: Opioid addiction is an ongoing problem in North Carolina. This study began with the goal of assessing the extent of the problem from a toxicological and epidemiological standpoint. This project will provide the toxicology community with statistics and valuable case studies relating to fentanyl overdoses in combination with heroin found in postmortem cases.

Method: Fentanyl, morphine, and 6-AM are detected as part of a routine LC/MS ion trap screening method. Confirmation and quantification analysis for these analytes is accomplished by LCMSMS. Scene findings, physical evidence, and urinary analysis for recent exposure to other opioids were considered when investigating the increase in deaths involving a combination of fentanyl and heroin.

Result: The decedents were divided into two groups: cases containing both heroin and fentanyl (usually in excess) and cases where non-pharmaceutical use of fentanyl was evident but no evidence of heroin was found. Several hypotheses emerged based on collected information and analytical findings. The heroin was laced with fentanyl, the decedents were taking both substances individually, or the decedents were coming down from a heroin high before injecting fentanyl. Illicit fentanyl use (based on case history, scene findings, physical evidence, etc.) was identified in 4 out of 123 total fentanyl cases in 2013, 61 out of 187 cases in 2014, and 18 out of 65 cases during the first quarter of 2015.

Case Study: Local law enforcement performed a field test on a powdery substance and found that it was positive for methamphetamine. After toxicology testing, both the physical evidence and decedent were positive for fentanyl. Despropionyl fentanyl, a by-product of the crude production of fentanyl, may explain the false positive kit reaction due to the secondary amine interaction with sodium nitroprusside.

Conclusion: The laboratory has observed a large increase in non-pharmaceutical grade fentanyl deaths, many of which are also positive for 6-AM and morphine. It is likely that the increase in potency when switching between these two opioids is problematic. It is unknown if dealers are selling the fentanyl as heroin or if the user is aware that he/she is injecting fentanyl. In addition, law enforcement agencies should be aware of the potential reaction of sodium nitroprusside test kits with by-products of non-pharmaceutical grade fentanyl. It is important to note that the issues addressed in this study are part of an on-going public health problem in North Carolina and will continue to be monitored by the North Carolina Office of the Chief Medical Examiner.

Keywords: Fentanyl, Heroin, Postmortem

S45

Finding the Fakes of Drug Testing; Detecting Sophisticated, Synthetic Urine Masquerading as Donor Samples

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Introduction: Multiple strategies are employed to conceal drug use when a motivated user must submit to urine drug testing. The practice of substituting synthetic urine for an authentic sample has recently become one of the more prevalent techniques to "beat" urine drug tests. Numerous brands of synthetic urine can be purchased via the internet or in paraphernalia shops. These products vary in composition, but most are designed to meet basic validity requirements of temperature, pH, specific gravity, creatinine levels, and color. Some products are significantly more complex in composition containing urea, uric acid, and even caffeine in an attempt to better represent a normal sample and reduce the likelihood of detection.

Objective: We set out to develop procedures capable of distinguishing biological samples from these sophisticated, artificial samples.

Method: Multiple sources of commercially available synthetic samples were procured and characterized using traditional tests for sample validity. Additionally, these samples were analyzed via UHPLC-TOF with the aim of identifying non-biological chemicals within the sample which could be utilized as clear indicators that a sample is of a synthetic source.
From the opposite perspective of the same problem, we evaluated the urine metabolome to identify constituents which should be present in all natural samples. Through published metabolomics databases and via UHPLC-TOF analysis we attempted to identify endogenous compounds which would be ubiquitously present in all natural samples at concentrations sufficient to allow relatively simple analysis techniques. Ideal validity markers would also be sufficiently esoteric as to impede manufacturers of synthetic samples from easily and cost effectively adding these chemical entities to their products. Once we identified likely markers we included them into a UHPLC-MS/MS procedure which also targeted metabolites of common xenobiotics. Greater than 1000 urine samples submitted for drug testing were then assayed.

Result: One brand of synthetic urine possessed a chemical entity within the product which would not be expected to exist within a natural sample, benzisothiazolone. This chemical compound was then found in multiple samples submitted for drug testing. Samples containing this compound met basic sample validity criteria; however, these samples possessed no other indication of being natural samples. Endogenous compounds were identified as promising candidates for new validity markers. These markers included a histidine metabolite, a norepinephrine metabolite, and a hemoglobin metabolite. Samples lacking the proposed validity markers but meeting standard validity criteria also lacked any other evidence of being natural samples. Samples possessing xenobiotic metabolites indicating their natural origin, also possessed the proposed validity markers.

Conclusion: We have successfully utilized esoteric endogenous markers as additional validity markers beyond the typical validity criteria. These markers appear to reliably identify natural samples and their absence reliably identified non-natural samples even when presented with sophisticated, artificial samples. After applying our proposed validity markers to a sampling of 1000+ samples submitted for urine drug testing, we believe approximately 2% of these samples are of synthetic origin. These synthetic samples detected using our proposed validity markers escaped detection using the standard validity criteria of creatinine, pH, and specific gravity.

Keywords: Synthetic Urine, Sample Substitution, Sample Validity

S46 ***"NIJ Funded"***

Presumptive Analysis of Electronic Cigarette Aerosol Using Solid-Phase Microextraction for Analysis by Gas Chromatography Mass Spectrometry (SPME-GC-MS) and Direct Analysis in Real Time AccuTOF™ Mass Spectrometry (SPME-DART-MS)

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Background/Introduction: Electronic cigarettes (e-cigs) have become popular to use as an alternative to traditional cigarettes, as a recreational activity, and as a delivery device for other licit or illicit drugs. These devices can be filled with a variety of refill formulations (e-liquids) that are typically comprised of nicotine, water, propylene glycol (PG), and glycerin (VG). Many of these e-liquids also contain a variety of flavoring and coloring agents, making them conceivably more appealing to a younger population of smokers. Few regulations are in place for the e-liquids or the constituents of the e-liquids, creating growing public controversy and criticism. Furthermore, little is known about the volatiles produced by these devices when the e-liquids are aerosolized, or "vaped." Therefore, an efficient and simple method that presumptively characterizes the aerosol produced by e-cigs is needed.

Objective: This study developed a screening method to use Solid-Phase Microextraction (SPME) for analysis by Gas Chromatography-Mass Spectrometry (GC-MS) and Direct Analysis in Real Time AccuTOF™ Mass Spectrometry (DART-MS) to characterize the volatiles produced in the aerosol of several commercial e-liquids.

Method: A simple trap was developed to capture the aerosol produced by an electronic cigarette. Ten commercial preparations of e-liquids from the United States were mechanically "vaped" into the trap from the electronic cigarette at 4.3V for 4 sec at 2.3mL/min. A SUPELCO polydimethylsiloxane (PDMS) SPME fiber was directly exposed to the aerosol stream for five seconds in order to adsorb any volatiles produced in the aerosol. Analysis was performed using an Agilent GC/MS 6890N/5973 Mass Selective Detector instrument with an HP-5MS column. Each sample was analyzed in splitless mode, with a 15 minute fiber desorption time. The initial temperature 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. Each sample was also exposed to the helium stream of a JEOL JMS T100LC AccuTOF™ Mass Spectrometer at 200 ^{0}C and collected in positive ion mode at 20, 30, 60, and 90V for orifice one. Needle voltage was set to 3500 V, with the discharge electrode at 150V, and the grid electrode at 250V. Limit of detection was administratively set to be 1.0mg/mL.

Result: SPME-GC-MS and SPME-DART-MS detected nicotine in all e-liquids labeled as containing nicotine. Furthermore, the additives propylene glycol and glycerin were found in all e-liquids as advertised. Flavoring agents were also detected, including carvone (spearmint), isoamyl alcohol (banana), benzaldehyde (almond), and limonene (citrus). No matrix interferences from PG or VG were detected.

Conclusion/Discussion: This study developed a method that allowed for the presumptive characterization of the volatiles produced by e-liquids during aerosolization in an electronic cigarette. The volatiles of the vapor phase were captured using SPME and analyzed using GC-MS and DART-MS. Several known constituents were detected, including nicotine, propylene glycol, glycerin, and a variety of flavoring agents.

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Keywords: Electronic Cigarettes, E-Liquids, Solid-Phase Microextraction

S47 ***"NIJ Funded"***

Simple, Rapid and Ultra-Sensitive Fluorescence Detection of Cocaine in Oral Fluid Based on Cooperative Binding Split Aptamer

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Background: Cocaine is one of the most abused drugs in the world. The identification and detection of cocaine is an urgent task for the guarding of social justice, public health and safety. Immunoassays have been widely used for sensitive and specific screening for cocaine in body fluids. Unfortunately, these antibody-based assays have disadvantages such as high cost, batch-to-batch variation and short shelf-life. Aptamers are single-stranded oligonucleotides, generated by Systematic Evolution of Ligands by EXponential enrichment (SELEX), that specifically bind with target molecules including proteins, small molecules and metal ions. Compared with antibodies, aptamers are synthetically produced and possess excellent stability with minimal batch-to-batch variability, and potentially offer low immunogenicity for in vivo applications. The selected cocaine binding aptamer has been proven to have a better specificity than antibodies. However, most current aptamer-based sensors have limited sensitivity due to the low affinity of the aptamer to its target.

Objective: To perform inexpensive, one-step, rapid and ultra-sensitive detection of cocaine in oral fluid samples, we here engineer a split aptamer and use this aptamer to perform cooperative binding based fluorescence for the detection of cocaine in oral fluid within 15 min at room temperature.

Method/Result: In this work, we innovatively engineered a cooperative binding split aptamer (CBSA) pair consisting of an unmodified long fragment and a fluorophore/quencher modified short fragment. Our CBSA contains two specific cocaine-binding sites. In the absence of cocaine, two CBSA fragments were seperated, the short fragment was single-stranded that held the fluorophore in close proximity to the quencher, generating very limited fluorescence. In the presence of cocaine, two cocaine molecules cooperatively assembled these two fragments of the CBSA, forming a cocaine/aptamer complex. The assembly greatly seperated fluorophore/quencher pair, resulting in a large fluorescent increase that can be monitored by a microplate fluorescence reader. Experimental results demonstrated that the two cooperative cocaine-binding sites in CBSA significantly increase the binding affinity and specificity of the aptamer for cocaine compared to the previously reported split aptamers containing a single-binding site. The limit of detection of 75 ng/mL was readily obtained in oral fluid samples within 15 minutes at room temperature, and we did not obtain any detectable cross reactivity against benzoylecgonine and ecgonine. In order to examine the specificity of our sensor, we prepared a sample set consisting of oral fluid samples that were spiked after collection with different concentrations of cocaine, cocaine interferences including ecgonine and benzoylecgonine. These samples were analyzed using a double blind approach. To validate our sensor, our results were then compared with existing immunoassays such as NEOGEN cocaine/benzoylecgonine ELISA kit.

Conclusion: We have developed a simple, inexpensive and highly sensitive fluorescent sensor for rapid and one-step detection of cocaine, as low as 75 ng/mL in oral fluid within 15 min at room temperature using only a set of CBSA pair. In comparison with NEOGEN® cocaine/benzoylecgonine ELISA kit, our method was completed in roughly one-tenth of the time required for ELISA (15 min vs. 3 hr) and showed comparable results in cocaine quantification in fortified oral fluids samples, with better specificity against benzoylecgonine and ecgonine.

Keywords: Cooperative Split Aptamer, Cocaine, Oral Fluid

S48 ***"NIJ Funded"***

Characterization of Electronic Cigarette Refill Formulations and Dose Capture of Nicotine in Aerosol by DART-AccuTOF™ MS, HPLC-MS/MS, and GC-MS

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Introduction: Electronic cigarettes (e-cigs) are a form of a nicotine delivery system that aerosolizes a solution, known as an e-liquid, which generally contains humectants, nicotine, and flavorant compounds. The internet is rife with information promoting the use of these devices to deliver illicit substances by adulterating the e-liquid formulations, modifying the devices, and varying power output. The media has reported news of controversy and criticism regarding their use and abuse. An analytical methodology to analyze the e-liquids and the aerosol generated by the devices is imperative to characterize the abuse potential in this growing industry.

Objective: The objective of this research was to characterize e-cig e-liquids and the aerosols by developing qualitative and quantitative methods of analysis by Direct Analysis in Real Time AccuTOF™ Mass Spectrometry (DART-AccuTOF™ MS), high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS), and gas chromatography mass spectrometry (GC-MS).

Method: Twenty-seven e-liquids with nicotine concentrations ranging from 0-22 mg/mL were screened using DART-AccuTOF™ MS (JEOL USA, Peabody, MA) with orifice 1 at 20 V for rapid analysis of their composition. Nicotine quantitation of the e-liquids was performed using an Applied Biosystems (Foster City, CA) 3200 QTRAP with a turbo V source for TurboIon Spray® attached to a Shimadzu (Columbia, MD) SCL HPLC with nicotine-d4 as an internal standard. The glycol composition of the e-liquids was determined using an HP 6890 GC/5793MSD (Santa Clara, CA) with a Stabilwax® (Restek Inc., Bellefonte, PA) column and 2,3-butanediol as internal standard for quantitation. The aerosols generated by the device were captured using a water trap constructed from common laboratory equipment, and the water was subsequently analyzed for nicotine using the same instrumental method as for the quantitation of nicotine in the e-liquids. The power supply on the electronic cigarette was varied from 3.7 to 4.7 volts.

Result: The DART-AccuTOF™ MS screening method was able to identify the accurate mass peaks resulting from the protonated molecular ion of nicotine within 5 mmu. Mass peaks resulting from the propylene glycol and/or glycerin solvent and potential additives such as flavorants were distinguished. Nicotine concentration ranged from 53-139% of the stated label concentration in the commercially obtained formulations. Glycol composition of the solvent ranged from pure propylene glycol to pure glycerin, and was generally accurate to the product description, with no toxic glycols detected. Nicotine concentrations in the aerosol trap generally increased with the voltage of the device.

Conclusion: The DART-AccuTOF™ MS was determined to be an effective instrument to screen for nicotine and other additives in e-cig formulations. The HPLC-MS/MS method identified and quantified nicotine in these formulations, showing a wide variation in concentrations relative to the labeled values. Glycol concentrations as determined by GC-MS were generally consistent as advertised on the label. The variance of actual nicotine concentrations indicates poor quality assurance and quality control within the industry. This study demonstrated an effective mechanism for the capture and analysis of aerosols which will be used in further studies to characterize the delivery of illicit substances and pharmaceuticals by e-cigs.

Keywords: Electronic Cigarettes, Nicotine, Aerosol

S49

Z-Drug Compliance: Urinary Sedative Hypnotic Metabolite Detection and Prevalence in Chronic Pain Patients

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Introduction: "Z-drugs" are regularly prescribed in the United States for sleep difficulties; however, little is known about their metabolite excretion in chronic pain patients. Zolpidem, zaleplon and eszopiclone are extensively metabolized *in vivo* and metabolite detection may provide improved accuracy for compliance determinations, thereby improving clinical decisions and treatment courses. To our knowledge, no reported method simultaneously quantifies zolpidem, eszopiclone (as zopiclone racemate), zaleplon and a metabolite for each in urine.

Objective: After attending this presentation, attendees will be able to describe an LC/MS/MS method for the simultaneous identification and quantification of zolpidem (Ambien®), eszopiclone (Lunesta®), zaleplon (Sonata®) and their primary human urinary metabolites, zolpidem 4-phenyl carboxylic acid (ZCA), N-desmethylzopiclone (NDZ) and 5-oxozaleplon (5-OZ), respectively. This presentation provides a novel analytical method for sensitive and specific simultaneous quantification of sedative hypnotic parents and metabolites in a single urine extract, as well as providing useful data detailing sedative hypnotic metabolite prevalence in a chronic pain patient population.

Method: This study was IRB-approved. Analytes were extracted from 1 mL human urine by salt-assisted liquid-liquid extraction followed by drying and reconstitution in 200 µL aqueous mobile phase. Samples were injected onto an LC/MS/MS instrument comprised of a Shimadzu Prominence HPLC and ABSciex API 3200 tandem mass spectrometer. Ionization was by electrospray (positive mode) with MRM mode employed for detection and quantification. Gradient chromatographic separation starting at 7% B (0.1% formic acid in acetonitrile) was achieved using a C_{18} column (100 x 2.1mm, 3 µm particle). Flow rate was 0.7 mL/min, with an increase to 1.2 mL/min mid acquisition with an overall run time of 4 minutes.

Result: Conservative limits of quantification (LLOQ) were 4 ng/mL for zolpidem, eszopiclone, zaleplon and ZCA and 10 ng/mL for 5-OZ and NDZ. The assay was validated for linearity from 4-1000 ng/mL for zolpidem, 4-5000 ng/mL for eszopiclone and zaleplon, 4-2500 ng/mL for ZCA and 10-5000 ng/mL for 5-OZ and NDZ ($r2 > 0.995$ and concentrations within ±20% of target). Inter-batch accuracy (bias) and imprecision (n=24) were 85-112% of target and 2.3-5.8% relative standard deviation, respectively.

A total 17391 directed-analysis urine samples were obtained from chronic pain patients over 9 months and analyzed. Results were de-identified and examined for prevalence of drugs and metabolites, with concentrations normalized to urine specific gravity. Zolpidem was detected > LLOQ in 4371 specimens (25.1%), while ZCA was detected in 7532 specimens (43.3%). Only 30 specimens (0.17%) contained zolpidem alone; 14 of these had zolpidem concentrations >1000 ng/mL. Eszopiclone was detected > LLOQ in 373 specimens (2.14%) while NDZ was detected in 401 specimens (2.31%). No specimens were identified containing eszopiclone parent only. Parent zaleplon was detected > LLOQ in only 2 specimens (6.0 and 6.9 ng/mL) although an additional 10 had zaleplon present > LOD (1 ng/mL). NDZ was not detected in any specimen.

Conclusion: An LC/MS/MS method for simultaneous detection and quantification of three sedative hypnotics and metabolites in human urine is presented. Addition of zolpidem and eszopiclone metabolite to compliance determinations resulted in substantially more positive samples compared to parent alone. This was not true for zaleplon, although analyte instability and lack of hydrolysis are possible factors in this finding. This method is rapid and conducive to a high-throughput environment. Improved detection windows for sedative hypnotic intake should prove useful in both clinical and forensic settings.

Keywords: Sedative Hypnotic, Compliance, LC/MS/MS

S50

Quantitation of Fentanyl Analogs in Dried Blood Spots by Flow-Through Desorption Coupled to Online Solid-Phase Extraction Tandem Mass Spectrometry

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Background/Introduction: The first automated dried blood spot analysis for fentanyl analogs was developed and assessed. Multiple fentanyl analogs have been developed with varying potencies for use in medical and veterinary fields, and illegal sales. Human exposure to fentanyl analogs is typically confirmed through the analysis of blood or urine. Dried blood spots (DBS) can overcome the challenges of whole blood shipment and storage while also providing small sample volumes.

Objective: A quantitative method was developed to detect fentanyl, sufentanil, carfentanil, alfentanil, lofentanil, α-methyl fenatnil, and 3-methyl fentanil in dried blood spots using an automated dried blood spot autosampler coupled with online solid-phase extraction tandem mass spectrometry (SPE-MS/MS). This method can confirm human exposures to these compounds with minimal sample volume and reduced shipping and storage costs for samples.

Method: Seven fentanyl analogs were detected and quantitated from 5µL dried blood spots using a dried blood spot autosampler coupled with online SPE-MS/MS. Isotopically labeled internal standards were used as available. Compounds were extracted from the DBS using flow through desorption with 15:85 Methanol: Water 1% formic acid and internal standards, which was loaded directly onto an online C18 SPE cartridge. The fentanyl analogs were then eluted using a three minute gradient and analyzed by MS/MS. This method was assessed for precision and accuracy using matrix matched calibrators and quality control samples.

Result: Extraction from the dried blood spot was optimized to produce the highest signal while maintaining retention on the SPE. Elution from the SPE cartridge provided adequate separation from possible matrix interferences while maintaining acceptable peak shape for each analyte. Matrix matched calibrators produced a linear curve with an R^2 value greater than 0.98 from 1 ng/mL to 100 ng/mL for all compounds. Evaluation of quality control materials showed high precision and accuracy for all compounds. Those with matched internal standards demonstrated slightly higher precision and accuracy.

Conclusion/Discussion: The developed method measures exposure to multiple fentanyl analogs in humans using dried blood spots. Automation of the DBS analysis reduced sample preparation time, allowed for fast and efficient desorption of the DBS, and use of full spot analysis corrected for hematocrit effect on analyzed volume. This method provides a fully automated means to detect and discern between seven fentanyl analogs with the benefits of using dried blood spots and acceptable precision and accuracy for detecting levels anticipated following overdose to these potent opioids.

The findings and conclusions in this study are those of the authors and do not necessarily represent the views of the U.S. Department of Health and Human Services, or the U.S. Centers for Disease Control and Prevention. Use of trade names and commercial sources is for identification only and does not constitute endorsement by the U.S. Department of Health and Human Services, or the U.S. Centers for Disease Control and Prevention.

Keywords: Dried Blood Spots (DBS), Fentanyl, Opioids

P01

Evaluation of Different Enzymes on Hydrolysis Efficiencies of Glucuronide Drug Metabolites in Human Urine

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Background/Introduction: A trend in recent years is that many prescription drugs are being increasingly diverted and abused by all age groups. Urine drug testing has thus become an important tool to monitor compliance and detect the presence of illicit drugs. The primary metabolites of many such drugs are glucuronides, which can be challenging to analyze due to their highly polar nature and poor ionization efficiencies. β-glucuronidase is commonly utilized for enzymatic hydrolysis of glucuronide metabolites to their parent drug. β -glucuronidase enzymes are derived from several sources including molluskan and *E. coli* sources.

Objective: Here we evaluate the hydrolysis efficiency of β-glucuronidase derived from *Patella vulgata* (limpet), *Helix pomatia* (snail), Red abalone, and *E. coli* on drugs of abuse in a synthetic urine matrix.

Method: A cocktail of glucuronide drug conjugates were spiked into a synthetic urine matrix along with isotopically labeled (deuterated) parent drug internal standard. Molluskan and *E. coli* enzymes were sourced from Sigma-Aldrich. Prior to running the hydrolysis experiments, enzymatic activities were verified using the standard phenolphthalein glucuronide colorimetric assay and based on this, enzyme dose (units/µL urine) was accurately normalized. Enzyme was added at 1 or 10 units/µL, pH was varied from 4-6 (molluskan) or 6-8 (*E. coli)*, and temperatures of 37 or 60 °C. Hydrolyzed samples were collected over time, followed by protein precipitation and analysis by LC-MS/MS.

Result: Molluskan-derived enzymes showed optimal performance at 60 °C, pH 5.0, while *E. coli* enzyme performed best at 37 °C, pH 6.8. Benzodiazepines, testosterone, and THC glucuronides tested were readily hydrolyzed even at lower enzyme titers. Opioid glucuronides tested generally required higher titers for complete hydrolysis within 1-2 hours, while codeine-6-β-D-glucuronide and morphine-6-β-D-glucuronide required higher titer and up to 6 hours for complete hydrolysis.

Conclusion/Discussion: Numerous glucuronidase enzymes are available for hydrolysis of metabolites in urine drug testing protocols. This work highlights the need for optimization of enzyme source, titer, pH, and temperature for various classes of drugs of abuse.

Keywords: β -glucuronidase, Glucuronide, Drugs of Abuse

P02
Performance of the NeoSal™ Oral Fluid Sample Collector

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Introduction: Oral fluid testing is an increasingly important and non-invasive tool for the routine monitoring of drugs of abuse. Several commercialized oral fluid collectors are available. Most collectors utilize an absorbent pad, sample volume adequacy indicator (SVAI) system, sample storage tube and sample stabilization/recovery buffer. NeoSal was designed with similar collector element features and was optimized for ease of use and recovery of several opiates, amphetamines, benzoylecgonine, PCP, Delta-9-THC and diazepam drugs from Oral Fluid**.**

Objective: This study describes in part some of the observed features of the NeoSal device and a comparison of drug recovery performance with three other commercially available collector systems.

Method: The absorption of oral fluid onto NeoSal device pad, SVAI activation reproducibility, and recovered diluted oral fluid volume following removal of the device pad from the NeoSal device were confirmed by using either a donor oral fluid sample pool or with Neogen EIA buffer (500mL CAT# 301276). Sample volume absorbed was determined gravimetrically using a NIST traceable Mettler analytical balance.

At a third party oral fluid drug testing lab, negative donor oral fluid pools were spiked with opiate family group (6-monoacetylmorphine, morphine, codeine, oxycodone, hydrocodone, hydromorphone and oxymorphone), amphetamine family group ((+) amphetamine, (+) methamphetamine, MDMA, MDA and MDEA), benzoylecgonine, PCP, or Delta-9-THC at SAMHSA confirmation specified cutoffs. Additionally, a spike of diazepam at 4 ng/mL was included. A 1 mL sample of the spiked oral fluid was applied directly to the device pads. The pads were then transferred to the storage tubes with sample stabilization/recovery buffer and incubated at 20-25°C overnight in darkened conditions. Aliquots of each sample were obtained following manufacturer instructions and samples were processed using solid-phase extraction, followed by LCMSMS analysis. Recovery analysis was calculated based on paired samples with no collection pad.

Result: The mean mass of oral fluid and EIA buffer absorbed into the NeoSal pad material was 730 mg +/- 70mg and 789mg +/- 2mg, respectively. The mean SVAI activation time for oral fluid and EIA buffer collection was 75.0 s +/- 36.7 s and 20 s +/- 1 s, respectively. The overall NeoSal device drug recoveries were similar to the three other commercial devices. Recoveries for the opiates group ranged from 88.1-94.2% for NeoSal and 47.6-117.3% for commercial devices 1-3. Recoveries for the amphetamine group ranged from 90.8-95.3% for NeoSal and 83.9-103.9% for commercial devices 1-3. Recovery for benzoylecgonine was 90.2% for NeoSal and 83.1-90.0% for commercial devices 1-3. Recovery for PCP was 93.1% for NeoSal and 84.0-97.0% for commercial devices 1-3. Recovery for Delta-9-THC was 82.8% for NeoSal and 40.3-83.9% for commercial devices 1-3. Recovery for diazepam was 81.5% for NeoSal and 79.3-88.0% for commercial devices 1-3.

Conclusion: Based on these results, the NeoSal Oral Fluid Collector device is sufficiently consistent in its ability to absorb oral fluid. As expected, real oral fluid sample collection timing had noticeably more variability when compared to EIA buffer. Overall drug recoveries were comparable or in certain cases higher than the three other commercial collection devices. Most noticeable device drug recovery differences were observed in opiates group and Delta-9-THC.

Keywords: NeoSal™, Oral Fluid, Collector, Drug Recoveries

P03

Recovery and Stability of Δ^9-Tetrahydrocannabinol Using the Oral-Eze® Oral Fluid Collection System and Intercept® Oral Specimen Collection Device[1]

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Introduction: Oral fluid (OF) is increasingly being used for clinical, forensic and workplace drug testing as an alternative to urine. Uncertainties surrounding OF collection device performance, drug stability, and testing reproducibility, may be partially responsible for delays in the implementation of OF testing in regulated workplace drug testing programs.

Method/Result: The stability of Δ^9-THC (THC) in spiked and authentic specimens was examined after routine collection, transport, and laboratory testing. Data were obtained from a collaborative study with initial testing performed by enzyme immunoassay and confirmation testing achieved using gas- and liquid-chromatography coupled to mass spectrometry. To determine THC stability using a realistic time course for OF collection and handling, THC-spiked OF (1.5 and 4.5 ng/mL) was applied to Oral-Eze devices, processed, and analyzed by LC-MS-MS (Waters 2795 Alliance HPLC/ Micromass® Quattro Micro triple quadrupole MS and Z-Spray electrospray ionization source operating in positive mode). Acceptable recovery and stability (85-104%) was observed after refrigerated (2-8°C) and room (21-25°C) temperatures tested at 7, 14, and 21 days. Neat OF samples collected with Oral-Eze, processed per the package insert, and spiked with THC at 3 and 6 ng/mL were used to investigate the stability of processed (diluted in buffer/preservative) OF samples containing THC at or above the cutoff (3 ng/mL, neat OF). Analysis by GC-MS (6890N Agilent GC with Deans Switch/5973 Agilent Mass Selective Detector) indicated Oral-Eze aliquots were stable (± 20%) at room temperature (21-25°C), refrigerated (2-8°C), and frozen (-25 to -15°C) conditions up to 1 month, while samples collected with Intercept devices showed decreases at refrigerated and room temperatures. After long-term refrigerated or frozen storage, maximum reductions in THC concentrations of 42% for Oral-Eze and 69% for Intercept were observed by GC-MS. Following ≥ 1 year frozen storage, 80.7% of authentic workplace drug testing specimens (n=130) positive by GC-MS for THC using a 3 ng/mL cutoff were reconfirmed positive (±25%), with an average THC decrease of 4.2%. Specimens (n=47) processed with Oral-Eze (diluted) and tested via enzyme immunoassay (CEDIA® Cannabinoids OFT Assay) were concordant with LC-MS-MS results and showed 100% sensitivity and 95% specificity. Paired specimens (n=472) collected with Oral-Eze and Intercept exhibited 98% overall agreement upon immunoassay analysis between the test systems.

Conclusion: Collectively, these data demonstrate consistent and reproducible recovery and stability of THC in OF after collection, transport and laboratory testing using the Oral-Eze OF Collection System.

Keywords: Oral Fluid Collection, Oral Specimen Collection Device, Oral-Eze®

[1] A subset of the data included in this manuscript were presented during a poster session at The International Association of Forensic Toxicologists (TIAFT) annual meeting in Buenos Aires, Argentina in 2014.

P04

Development of Homogeneous Enzyme Immunoassays for the Detection of Amphetamine, Cocaine and Metabolites, Opiates, and PCP in Oral Fluid Using the Intercept® *i2he*™ Oral Fluid Collection Device

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Introduction: Because of the issues associated with the collection of urine and with increased use of sophisticated urine adulterants, oral fluid is gaining acceptance as the sample matrix for drug of abuse testing. SAMHSA proposed guidelines in April 2004 to utilize oral fluid for the detection of illicit drugs in workplace drug testing programs. Oral Fluid as a sample for drug of abuse testing has several advantages. Unlike urine, oral fluid sample collection is less invasive and no special facilities are required. Since the oral Fluid samples are collected under observation, there is minimal risk of sample adulteration or substitution.

Objective: The objective of this study is to develop Automated homogeneous enzyme immunoassays for the detection of *d*-amphetamine, cocaine and cocaine metabolites, opiates, and PCP in oral fluid collected with the Intercept® *i2he*™ Oral Fluid Collection Device.

Method: The Intercept® *i2he*™ Oral Fluid Assays use recombinant DNA technology to produce a unique homogeneous enzyme immunoassay system based on the bacterial enzyme β-galactosidase. The Intercept® *i2he*™ Amphetamine, Cocaine, Opiate, and PCP Oral Fluid Assays have neat oral fluid cutoffs of 150 ng/mL, 15 ng/mL, 30 ng/mL, and 3 ng/mL respectively. The assays utilize a multi-drug set of calibrators and controls that are liquid ready-to-use. The Intercept® *i2he*™ Oral Fluid Collection Device is equipped with a sample adequacy indicator that turns blue after the collection of 1.0 +/-0.1mL of oral fluid. The 1.0 mL of oral fluid is diluted with 2.0 mL of preservative buffer.

Result: The performance of each assay was evaluated on the Beckman Coulter AU480 analyzer. A randomized CLSI 20-day precision was followed with two replicates of each sample for each run, two runs per day for twenty non-consecutive days, total N= 80/level. Samples for each assay were prepared at 25% intervals from 75% below the cutoff (100%) to 100% above the cutoff and the oral fluid spikes were processed using the collection device. The precision study showed acceptable results by detecting ≥ 95% of the negative spiked samples as negative and ≥ 95% of the positive spiked samples as positive. The Amphetamine Assay, which was calibrated with d-amphetamine, showed 1%, 8% and 2%, cross-reactivity to *l*-Amphetamine, Phenylethylamine, Phentermine respectively. The Cocaine Assay, which was calibrated with BE, demonstrated 95% cross-reactivity to cocaine. The Opiate Assay, which was calibrated with morphine, demonstrated ≥ 80 % cross-reactivity to hydrocodone and 6-acetylmorphine. The low control and high control were detected accurately in the presence of endogenous and exogenous substances indicating that there was no interference in the assay from these substances. There was no significant cross-reactivity from over the counter medications or other concomitantly used drugs. For each assay, eighty authentic neat oral fluid samples, including a minimum of eight near cutoff samples, were tested by immunoassay and the results showed ≥95% agreement with LC-MS/MS. Intercept® *i2he*™ Oral Fluid Collection Device demonstrated amphetamine, BE, morphine, and PCP recovery of ≥95% and stability for 21 days at 37°C.

Conclusion: The Intercept® *i2he*™ Oral Fluid Assays demonstrated excellent specificity and sensitivity to *d*-amphetamine, cocaine and BE, morphine, and PCP. The Intercept® *i2he*™ Oral Fluid Collection Device demonstrated excellent drug recovery and stability. The assay can be applied to various clinical chemistry analyzers.

NOTE: These Assays are not currently approved by FDA

Keywords: *d*-Amphetamine, Cocaine, Benzoylecgonine, Morphine, Opiate, Phencyclidine, Oral Fluid, Immunoassay

P05

Development of Homogeneous Enzyme Immunoassays for the Detection of Cannabinoids and Methamphetamine in Oral Fluid Using the Intercept® *i2he*™ Oral Fluid Collection Device

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Introduction: Because of the issues associated with the collection of urine and with increased use of sophisticated urine adulterants, oral fluid is gaining acceptance as the sample matrix for drug of abuse testing. SAMHSA proposed guidelines in April 2004 to utilize oral fluid for the detection of illicit drugs in workplace drug testing programs. Oral Fluid as a sample for drug of abuse testing has several advantages. Unlike urine, oral fluid sample collection is less invasive and no special facilities are required. Since the oral Fluid samples are collected under observation, there is minimal risk of sample adulteration or substitution.

Objective: The objective of this study is to develop Automated homogeneous enzyme immunoassays for the detection of cannabinoids and *d*-methamphetamine in oral fluid collected with the Intercept® *i2he*™ Oral Fluid Collection Device.

Method: The Intercept® *i2he*™ Oral Fluid Assays use recombinant DNA technology to produce a unique homogeneous enzyme immunoassay system based on the bacterial enzyme β-galactosidase. The Intercept® *i2he*™ Cannabinoids and Methamphetamine Oral Fluid Assays have neat oral fluid cutoffs of 3 ng/mL and 120 ng/mL respectively. Each Assay has its own set of calibrators and controls that are liquid ready-to-use. The Intercept® *i2he*™ Oral Fluid Collection Device is equipped with a sample adequacy indicator that turns blue after the collection of 1.0 +/-0.1mL of oral fluid. The 1.0 mL of oral fluid is diluted with 2.0 mL of preservative buffer.

Result: The performance of each assay was evaluated on the Beckman Coulter AU480 analyzer. A randomized CLSI 20-day precision was followed with two replicates of each sample for each run, two runs per day for twenty non-consecutive days, total N= 80/level. Samples for each assay were prepared at 25% intervals from 75% below the cutoff (100%) to 100% above the cutoff and the oral fluid spikes were processed using the collection device. The precision study showed acceptable results by detecting ≥ 95% of the negative spiked samples as negative and ≥ 95% of the positive spiked samples as positive. The methamphetamine assay, which is calibrated with d-methamphetamine, showed 80% cross reactivity to MDMA and 11% cross-reactivity to *l*-methamphetamine. The low control and high control were detected accurately in the presence of endogenous and exogenous substances indicating that there was no interference in the assays from these substances. There was no significant cross-reactivity from over the counter medications or other concomitantly used drugs. For each assay, eighty authentic neat oral fluid samples, including 10 near cutoff samples for THC and 26 near cutoff samples for methamphetamine, were tested by immunoassay and the results showed ≥95% agreement with LC-MS/MS for both assays. The Intercept® *i2he*™ Oral Fluid Collection Device demonstrated THC recovery of 96% and methamphetamine recovery of 92% and stability for 21 days at 37°C for both assays.

Conclusion: The Intercept® *i2he*™ Oral Fluid Assays demonstrated excellent specificity and sensitivity to Δ^9 THC and its metabolites and to d-methamphetamine and its metabolites. The Intercept® *i2he*™ Oral Fluid Collection Device demonstrated excellent drug recovery and stability in both assays. The assay can be applied to various clinical chemistry analyzers.

NOTE: These Assays are not currently approved by FDA

Keywords: Cannabinoids, Δ^9 THC, *d*-Methamphetamine, Oral Fluid, Immunoassay

P06

Development of a New Homogeneous Enzyme Immunoassay for the Detection of Buprenorphine, Norbuprenorphine and their Glucuronides in Human Urine

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Background/Introduction: Buprenorphine is a semi-synthetic opioid derived from thebaine, and is used clinically as a substitute therapy for opiod dependence. Subutex® and Suboxone® are the commonly prescribed drugs containing buprenorphine. Buprenorphine is metabolized into norbuprenorphine, and both buprenorphine and norbuprenorphine are conjugated to glucuronide to become buprenorphine-glucuronide and norbuprenorphine-glucuronide. Commercially available immunoassays either detect only Buprenorphine and Buprenorphine glucuronide, or detect only only Buprenorphine and Norbuprenorphine.

Objective: The objective of this study was to develop a new homogeneous enzyme immunoassay that could detect buprenorphine and norbuprenorphine and their metabolites in urine using the CEDIA® technology. Further, this study is to utilize a new monoclonal antibody with minimal cross-reactivity to opiate compounds or other structurally unrelated compounds. The ability to detect metabolites, including the glucuronides, by an immunoassay will minimize the occurence of false negatives and improve specificity and sensitivity of the assay.

Method: CEDIA® technology is based on the bacterial enzyme β-Galactosidase which has been genetically engineered into two inactive fragments, Enzyme Acceptor (EA) and Enzyme Donor (ED). These fragments spontaneously re-associate to form an active enzyme. In the absence of analyte from the sample, the specific antibody binds the ED-drug conjugate causing a decrease in enzyme activity. The free drug in the sample will compete for the limited number of antibody binding sites, making the ED-drug conjugate available for complementation to form an active enzyme. This phenomenon creates a direct relationship between the drug concentration in urine and enzyme activity. The enzyme activity is then determined spectrophotometrically at 570 nm. The performance of the assay was evaluated on the Beckman Coulter AU680 analyzer. The Assay uses a 10 ng/mL cutoff calibrator with controls at ± 25% of the cutoff. The reagents are lyophilized and the calibrators and controls are liquid ready-to-use. Patient samples were obtained from pain management laboratories. The samples were analyzed by LC-MS/MS to determine the levels of buprenorphine, norbuprenorphine, buprenorphine-glucuronide and norbuprenorphine-glucuronide. Precision, method comparison, cross-reactivity, spike recovery and stability studies were performed to determine the overall performance of the assay.

Result: Using 10 ng/mL as the cut-off calibrator, the selected monoclonal antibody is specific to buprenorphine, norbuprenorphine and their respective glucuronides, with 100% cross-reactivity to buprenorphine, 130% cross-reactivity to norbuprenorphine, 77% cross-reactivity to buprenorphine-glucuronide, and 87% cross reacitivity norbuprenorphine-glucuronide. The antibody has no significant cross-reactivity to other opiate compounds (such as codeine or nalorphine) or structurally unrelated compounds, such as mitragynine, amisulpride, sulpiride, chloroquine and hydroxychloroquine. Nine (9) levels of precision spikes were prepared at ± 25% increments or decrements from the 10 ng/mL cut-off calibrator (0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5 and 20 ng/mL). Precision study using these nine levels demonstrated excellent precision, in both qualitative and semi-quantitative modes, with no cross-over between the levels. Method comparison study using 285 urine samples showed > 90% agreement between immunoassay and LC-MS/MS. Reconstituted reagents stored at 2-8^{0}C or on-board clinical analyzer were stable for up to 60 days. Spike recovery studies showed good recovery as confirmed by LC-MS/MS.

Conclusion: The preliminary data on the CEDIA® Buprenorphine II Assay indicates excellent specificity and sensitivity to buprenorphine and its metabolites, without any significant cross-reactivity to other commonly abused opiate compounds.

NOTE: The assay is currently in development and is not approved by FDA

Keywords: Buprenorphine, Metabolites, Immunoassay, CEDIA®

P07
The 6-AM Conundrum

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Background: 6-acetyl morphine (6-AM) in biological fluids has long been considered an indicator of heroin use. Recently, the presence of 6-AM in patient cases where heroin abuse is unlikely has caused the industry to question whether the compound could be produced by other means. Some possible sources suggested include pharmaceutical morphine contamination; co-administration with an acetylating drug like acetylsalicylic acid; analytical generation through extraction or analysis; interference from 3-acetyl morphine (3-AM); and *in vivo* production. In December, 2014, a postmortem case was identified with 32,000 ng/mL morphine in central blood and 2.5 ng/mL 6-AM. The 6-AM finding was suspicious because the patient had been in hospice prior to death. Seven other cases from 2014 were identified with morphine concentrations above 10,000 ng/mL. This triggered an investigation to determine whether the analytical method had produced 6-AM as an artifact.

Objective: This presentation will impact the forensic science community by performing a critical review of all existing research, as well as conducting additional experiments to determine if 6-AM can be produced by any mechanism beyond heroin use.

Method: Free opiates were prepared by solid phase extraction and quantitated on a Waters TQS Tandem Mass Spectrometer with a Waters Acquity Ultra Performance Liquid Chromatography (LC-MS/MS) system using a BEH C18 column. The reporting limit of 6-AM on this procedure is 1.0 ng/mL. Un-extracted morphine was fortified into mobile phase at high concentrations to confirm purity. Neat injections were prepared by spiking into elution solvent or spiking directly into the reconstitution step of the extraction. Next, blood was spiked with 10,000 ng/mL morphine with and without incubation at 37°C for two hours. Multiple blank blood lots and preservatives were spiked at decreasing morphine concentrations to determine where the 6-AM analyte becomes detectable.

Result: Morphine samples spiked into elution solvent as well as the morphine samples spiked directly into the reconstitution solvent did not produce positive results for 6-AM. A small response was observed below the reporting limit. Morphine spiked into blood at 10,000 ng/mL with or without incubation at 37°C for two hours produced detectable 6-AM. 3-AM spiked in blood at 500 ng/mL produced a result for morphine at 370 ng/mL and 6-AM at 1.0 ng/mL as well as traces of codeine and oxymorphone.

Discussion: Neat injections of morphine at high concentrations did not produce an artifact for 6-AM by this method. The presence of 6-AM in the spiked blood samples is interesting considering the same lot and vial of morphine standard was utilized for all experiments and it had already been proven to be free of 6-AM. Percentages of 6-AM in the blood samples compared to morphine area counts may actually be smaller than those calculated due to the saturation of morphine through the analytical column and detector. More work is being performed on this aspect as a portion of phase three. The 3-AM results are also very interesting. Having the same molecular weight, this isomer of 6-AM may co-elute and be analytically indistinguishable from 6-AM, and in fact this may be the case with the small amount that was detected from the spiked blood sample at 500 ng/mL.

Keywords: Heroin, Morphine, Artifact

P08

Implementation of a High-Resolution Orbitrap Mass Spectrometer for Quantitative Analysis of Five Barbiturates in Urine

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Background/Introduction: Conventionally, forensic toxicology laboratories have used triple quadrupole mass spectrometers for LC-MS/MS analysis of barbiturates in biological matrices. High-resolution mass spectrometers should also be considered because of their versatile application range: screening, quantification and unknown identification.

Objective: We evaluated the performance of a Thermo Scientific™ Q Exactive™ Focus high-resolution hybrid quadrupole-Orbitrap mass spectrometer for quantitative analysis of 5 barbiturates in urine samples.

Method: Urine samples were spiked with internal standards (deuterated analogs) and diluted 20-fold with water. A10-µL aliquot was injected onto a Thermo Scientific Accucore™ C18, 2.6 µm, 50 x 2.1 mm fused core column. Analytes were separated in 6.0 min under gradient conditions using 5 mM ammonium acetate in water as mobile phase A and acetonitrile as mobile phase B. Detection was performed with a Q Exactive Focus mass spectrometer equipped with heated electropsray (HESI) source. Full scan MS2 spectra were collected for all analytes individually. For each analyte, two fragment ions were selected from the spectral data for data processing. Chromatograms were reconstructed with 5 ppm mass accuracy, and ion ratios were calculated for confirmation.

The calibration standards in synthetic urine (range 5-2000 ng/mL) and QC samples (25, 200, and 1000 ng/mL) were prepared in-house. Method precision, limits of quantitation (LOQ) and linearity ranges were obtained 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 urine samples from 7 donors at concentrations of 10, 25 and 100 ng/mL and comparing signals of analytes to those of samples prepared in water. Additionally internal standards %recovery was calculated in 48 urine donor samples analyzed with this method. Method performance evaluation data were compared to data collected with a triple quadrupole mass spectrometer.

Result: Limits of quantitation (defined as the lowest concentrations that had back-calculated values within 20%, RSD for 5 replicates within 20%, and ion ratio within required range) were 5 ng/mL for Amobarbital, Butalbital, Pentobarbital, Secobarbital and 25 ng/mL for Phenobarbital. The upper calibration range for all analytes was 2000 ng/mL with R^2>0.99. Analyte precision (RSD) for all calibration standards was better than 15.2% (inter-assay) and for QCs was better than 7.9% for intra-assay and 9.7% for inter-assay. Limited matrix effects were observed. Peak area recovery in spiked donor urine samples were in the range of 83.5-111%. Internal standard recoveries calculated against mean value in calibration standards were in the range of 76-108%. Carryover at the upper limit of quantitation (2000 ng/mL) was not observed. Method performance, including LOQ's, precision, accuracy and matrix effects, was comparable to that achieved with a triple quadrupole mass spectrometer.

Conclusion/Discussion: The method performance evaluation results indicate that Orbitrap-based mass spectrometers can be used for multi-drug quantitative methods. Method performance evaluation data indicates that the presented method is a good candidate for full validation in a toxicology laboratory.

Keywords: Barbiturates, Orbitrap, Quantitation

P09

Multi-Channel LC-MS/MS Forensic Methods for High-Throughput Screening to Detect Buprenorphine and Ethanol Use

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Background: Forensic laboratories are looking for high-throughput LC-MS solutions to lower per-sample analysis cost. Multi-channel LC systems improve system throughput by efficient utilization of the mass spectrometer's time, making the analytical workflow more cost-efficient.

Objective: We evaluated the performance of a new 4-channel LC system (LX-4) utilizing positive-displacement pumps for improved performance when compared to conventional reciprocating pumps. The LX-4 system was coupled to a Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer. Two analytical methods were used for evaluation experiments: analysis of buprenorphine/norbuprenorphine and analysis of ethyl glucuronide (EtG)/ethyl sulfate (EtS), both in urine.

Method: For analysis of buprenorphine and norbuprenorphine, live-donor urine specimens, calibrators (5 to 500 ng/mL) and quality control samples (QCs) were hydrolyzed by incubation with β-glucuronidase enzyme and then mixed with cold methanol containing buprenorphine-D_3 and norbuprenorphine-D_4 internal standards. The column used was a Thermo Scientific™ Accucore™ RP-MS, 2.6 µm, 50 x 2.1 mm. Mobile phases A and B were 0.1% formic acid in water and methanol, respectively. For analysis of EtG and EtS, live-donor urine specimens, calibrators (100-5000 ng/mL) and QCs were diluted 1:10 with water containing internal standards, EtG-D_5 and EtS-D_5. The column used was a Thermo Scientific™ Syncronis™ aQ 3 µm, 100 x 3 mm. Mobile phase A was water containing 0.1% formic acid and 2% methanol. Mobile phase B was methanol with 0.1% formic acid. After preparation, 20-µL aliquots of both sets of compounds were injected into one channel and across all channels.

Result: A maximum throughput of 34 urine samples per hour was achieved when batches were submitted across three channels. Since the data windows of both methods were a little more than one third of the total run time, adding the fourth channel did not increase sample throughput. However, using all four channels provided assurance that the total throughput would not be compromised in the event of one channel shutting down because of a leak or a column reaching its maximum pressure. The calibration curves collected with the buprenorphine/norbuprenorphine method were consistently linear ($r^2 > 0.995$, 1/X weighting) whether the calibrators were injected into one channel or across all channels. The calibration curves collected for EtG and EtS were consistently linear ($r^2 > 0.990$, 1/X weighting) whether calibrators were injected into one channel or across all channels. For both methods, internal standard peak areas showed less than 25% coefficient of variation (CV) among calibrators, QCs and specimens (n = 20) on any of the four channels. Retention time variations throughout these batches were less than 3% CV. Results were within +/- 15% of those determined on a conventional multichannel system using reciprocating pumps, which showed similar performance of linearity and reproducibility for both methods. Since the positive-displacement pumps of the new LX-4 system do not run continuously as do conventional pumps, they reduced solvent consumption at least 65% while running batches for both methods. The manner in which the new LX-4 system pressurizes the columns immediately before the injections resulted in reproducible retention times and peak shapes for each analyte; it eliminated the need for continuous flow through the columns between injections.

Conclusion: The new LX4 LC system utilizing unique positive-displacement pumps produced results for buprenorphine/norbuprenorphine and EtG/EtS batches that were consistent with those produced by the conventional 4-channel LC system utilizing reciprocating pumps. Comparatively, the multichannel system with positive-displacement pumps reduced solvent consumption by at least 65%. Other benefits included: smaller footprint when compare to conventional LX4 system, avoidance of pulsations of reciprocating pumps, system ease of use including operation, purging and maintenance.

Keywords: Multi-Channel LC Systems

P10

A Novel and Fast Workflow for Forensic Toxicological Screening and Quantitation Using a Quadrupole-Time-of-Flight LC-MS/MS System

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Introduction: (*For research use only, not for use in diagnostic procedures*) Forensic toxicological screening is challenging in that: (1) the target compound list can exceed hundreds with drastically varying chemical properties, (2) new compounds are constantly emerging; not detected by targeted analytical approaches, (3) common detection techniques (e.g. immunoassay) lack flexibility to adaption of new analytes, and (4) these same techniques often yield significant false-positive and false-negative rates. An alternative technique that is more sensitive and specific is therefore required for efficient screening and quantification.

Objective: We aimed to develop a sensitive and selective forensic toxicological screening workflow by utilizing a Quadrupole-Time-of-Flight mass spectrometer and novel MS/MS^{All} in an information independent acquisition approach; Sequential Windowed Acquisition of all Theoretical Masses (SWATH™) .

Method: Blank human urine samples were spiked with more than 50 common drugs, they were diluted 10-fold in 10% methanol, centrifuged and 10 µL sample was injected on the accurate mass LC-MS/MS system. HPLC separation was performed on reverse-phase columns at 30 °C. Two different LC gradients (6.5-min and 2-min) were developed. Data was collected on a TripleTOF® 5600+ mass spectrometer with Analyst® TF software 1.7 using the following conditions: For MS/MS^{All} acquisition, a comparison was made between using a fixed precursor isolation window width (40 Da) or variable isolation widow. The source (DuoSpray) parameters were: 2500 V spray voltage, 35 psi curtain gas, 600 °C source temperature, 60 psi for nebulizing and desolvation gas. Data was processed in MasterView™ software 1.1. Quantitation was performed with MultiQuant™ software 3.0.

Result: For screening and identification purposes both the 6.5 and 2.0 minute LC gradients using MS/MS^{All} acquisition were tested using multiple screening confidence criteria that included mass accuracy (less than 5 ppm), retention time error (less than 3%), MS/MS library matching (better than 30%) and combined score (better than 55%). The availability of MS/MS information for positive identification was required in this study. Due to better LC separation and reduced matrix affects, the 6.5 minute method yielded slightly better MS/MS library matching score than the 2 minute method. For the MS/MS^{All} workflow there were no false positives reported based on the listed criteria. There were 10 false negatives due to the absence of unmatched MS/MS information. Overall the analysis resulted in a detection rate of 94% with average library 'Fit' score of 95.2%. Compared to data using MS/MS^{All} acquisition with fixed window (90% detection), the detection rate using MS/MS^{All} acquisition with variable window was significantly improved, proving the importance of improved MS/MS selectivity information. For purposes of quantification and confirmation, overall the MS/MS mode provided better limit of detection amid higher specificity over the TOF-MS. This was achieved due to the observed cleaner LC chromatograms and/or lower baseline in MS/MS mode with selective fragment ion extraction. In MS/MS mode, SWATH™ acquisition ensures that all data is acquired at all times. Further, a novel variable-SWATH™-window approach was used to improve the MS/MS selectivity resulting in sensitive and unambiguous quantitation using fragment ions. Compounds that clearly benefited from the variable SWATH™ window acquisition included (but were not limited to) the following compounds: 6-MAM, codeine, EDDP, hydrocodone, hydromorphone, MDA, methadone, naloxone, norcodeine and norhydrocodone. As example for quantitative performance the calibration curves of imipramine, and sufentanil, in diluted urine at 40, 100 and 300% of cutoff concentrations resulted in linear fit regression with r value at 0.99999 and 0.99924 respectively.

Conclusion: A sensitive and selective workflow was developed for forensic toxicological drug screening using the TripleTOF® system. The novel MS/MS^{All} acquisition when used for screening achieved 95% detection rate in urine samples using a novel SWATH™ (variable window) data acquisition. With traditional TOF-MS-Information Dependent Aaquisition-MS/MS, quantitation can only be performed from TOF-MS mode but not from the in situ sporadic triggered TOF-MS/MS data points. It was shown in this study that, due to the continual and looped MS/MS scan function and better selectivity with the fragment ion information, SWATH™ acquisition enabled more sensitive detection in MS/MS mode of lower concentration species in complex matrices.

Keywords: Rapid Drug Screening, High Resolution Mass Spectrometry, Data Independent Acquisition

P11 ***"NIJ Funded"***

Drug Screening by Paper Spray Mass Spectrometry: A Direct Analysis Approach

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Introduction: There is a need for developing simpler methods to perform drug screening and quantitation in biological matrices by mass spectrometry, particularly for forensic applications such as post-mortem toxicological analysis. Paper spray mass spectrometry (MS) is a recently described method for the direct analysis of small molecules from biofluids such as blood and urine. The analysis is rapid, requires no sample preparation, and can detect drugs directly from dried blood spots at low or sub ng/mL levels when coupled to a triple quad mass spectrometer. Because there is no chromatography, the selectivity of this approach is lower than HPLC-MS/MS. It may have significant value as a screening method, however, due to its speed and simplicity relative to traditional HPLC-MS.

Objective: The objective is to evaluate the use of paper spray MS as a method for rapidly screening post-mortem blood samples. In this study, we focus on detection limits and selectivity.

Method: Analysis is performed by depositing blood onto a porous substrate such as paper and allowing it to dry. The paper comes precut and contained in a disposable cartridge. After the sample is dry, a solvent (typically 95% methanol with 0.01% acetic acid) is applied to the paper where it flows through the dried matrix spot by capillary action, performing an extraction as it flows through. A high voltage (3-4 kV) is applied to the moist paper, which generates gas phase ions of the analyte molecules from the tip of the paper via electrospray ionization followed by detection by a mass spectrometer. All of the analytical steps (except sample addition) are performed by an automated front end installed on the mass spectrometer (Prosolia, Inc. Indianapolis, IN). The mass spectrometer used in this study was a Thermo TSQ Vantage triple quadrupole. The instrument was operated in SRM mode with two fragment ions monitored for each target compound. Positive identification of a drug is made when both 1) the signal intensity threshold of the quantifier SRM transition exceeds a predetermined threshold and 2) the ratio of the quantifier SRM transition and a secondary qualifier SRM transition are within +/- 25% of the expected value.

Result: A list of 154 drugs and drug metabolites, along with screening cut-off values, was compiled in collaboration with toxicologists at AIT Laboratories (Indianapolis). The detection limits obtainable by paper spray MS/MS from spiked blood samples for a representative initial subset of the drugs will be presented (screening cutoff in ng/mL in parenthesis): amphetamine (50), buprenorphine (1), ketamine(100), gabapentin (500), fluvoxamine (20), butabarbital (500), alprazolam (5), furosemide (1000), cocaethylene (50), fentanyl (1), morphine (20), and zolpidem (50). The detection limits obtained from spiked blood samples were less than the cutoff for all of the drugs except zolpidem.

Selectivity of paper spray MS/MS will also be reported for this subset of drugs. A list of relatively common compounds that have the same nominal mass as the targets, and therefore could result in false positives, was compiled. Potential for interference was assessed based on several criteria: the probable concentration of the interfering compound relative to the target cutoff, the ionization efficiency of the interfering compound compared to the target, and the MS/MS spectra. To use gabapentin as an example, the potentially interfering compounds tested were rasagiline and metronidazole. In this case, neither of these compounds interfere with gabapentin because their product ion distribution is distinct. In other cases, similar product ions were generated upon collision induced dissociation and identification by MS/MS is more ambiguous. One example of this type is morphine; norcodeine and hydromorphone, which are closely related structural isomers to morphine, gave similar product ions to morphine, albeit in different relative intensities.

Conclusion: Preliminary data indicate that paper spray MS/MS has good potential as a drug screening method in forensic toxicology. Detection limits are below the desired cutoff for nearly all of the target compounds and selectivity from MS/MS is normally adequate with the exception of closely related structural isomers.

Keywords: Dried Blood Spots, Toxicology, Ambient Ionization

P12
Detection of Synthetic Cannabinoids in Two E-Cigarette Liquids

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Introduction: E-cigarettes are battery operated vaporizers that heat liquid solutions (e-liquids) and create an aerosol vapor instead of cigarette smoke. These e-liquids typically contain a combination of propylene glycol, glycerin, nicotine, and/or various flavorings. Though there have been no reports published in scientific literature regarding the presence of illicit substances in e-liquids, the media has reported that the e-cigarette has become a new delivery mechanism for various illicit substances.

Various synthetic cannabinoids were detected in two e-liquids sold in a local smokeshop by ultra-performance liquid chromatography with electrospray ionization time of flight mass spectrometry (UPLC/ToF) and ultra-performance liquid chromatography with electrospray ionization tandem mass spectrometry (UPLC/MS/MS).

Objective: Anecdotally synthetic cannabinoids have been reported as constituents or adulterants in various e-liquids. No published reports exist on this subject. The objective of this study was to obtain and analyze two e-liquid specimens for synthetic cannabinoids via UPLC/ToF and UPLC/MS/MS.

Method: Screening Analysis: The specimens were subjected to a solvent extraction with acetonitrile, vortex mixed for 3 minutes, centrifuged for 3 minutes at 3,000 RPM, and diluted 1:1,000 with an acetonitrile:DI water mixture. The solution was transferred to a vial for UPLC/ToF analysis.

Confirmatory Analysis: The specimens were prepared via a liquid-liquid extraction into hexane:ethyl acetate (98:2) at pH 10.2, vortex mixed for 2 minutes, and centrifuged for 2 minutes at 3,000 RPM. The organic layer was transferred to a new tube and evaporated to dryness under nitrogen gas flow. The residue was reconstituted in an acetonitrile:DI water mixture and the solution was transferred to a vial for UPLC/MS/MS analysis.

Result: The first e-cigarette liquid specimen was named Buzz Juice Liquid Incense and consisted of 5 mL of a white-colored liquid. The liquid had no odor. It screened positive for five synthetic cannabinoids: 5F-PB-22, AB-CHMINACA, AB-PINACA, ADB-PINACA, and MAB-CHMINACA. Quantitative analysis by UPLC/MS/MS revealed 5F-PB-22 (<0.2 ng/mL), AB-CHMINACA (22,733 ng/mL), AB-PINACA (4,568 ng/mL), ADB-PINACA (164 ng/mL), and MAB-CHMINACA (2,134,562 ng/mL).

The second e-cigarette liquid specimen was named Buzz Relax E-Juice and consisted of 5 mL of a yellowish-brown colored liquid. The liquid had an odor similar to either maple syrup or rum. It screened positive for four synthetic cannabinoids: 5F-PB-22, ADB-PINACA, PB-22, and XLR11. Quantitative analysis by UPLC/MS/MS revealed 5F-PB-22 (<0.2 ng/mL), ADB-PINACA (0.2 ng/mL), PB-22 (<0.2 ng/mL), and XLR11 (6 ng/mL).

Conclusion: The use of e-cigarettes and corresponding e-liquids has become more widespread over the past few years. It has been reported in media that the e-cigarette has become an avenue for administration of illicit substances. Several synthetic cannabinoid compounds were detected by UPLC/ToF and UPLC/MS/MS at varying concentrations in two e-liquid specimens that were available in a local smokeshop.

Keywords: Synthetic Cannabinoids, Electronic Cigarette, E-Liquid, UPLC/ToF, UPLC/MS/MS

P13
Short-Term Stability of Gamma-Hydroxybutyrate (GHB) in Oral Fluid

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Background/Introduction: Gamma-hydroxybutyrate (GHB) is a short-chain fatty acid endogenous to mammalian tissues. As GHB has been predominantly studied in blood and urine, there is limited information regarding endogenous concentrations in oral fluid or the stability of GHB in this matrix. De Paoli *et al* found endogenous concentrations of GHB in oral fluid ranging from 0.15 – 3.33 mg/L (median 1.13 mg/L) (1). Oral fluid is no longer considered an alternative matrix, and is frequently collected when monitoring an individual's compliance with drug treatment programmes, in workplace drug testing programmes as well as in roadside testing. Collection of oral fluid is non-invasive, and can be carried out with ease without specialist collection facilities. Studies conducted in Germany and Sweden, showed drivers with elevated concentrations of GHB following arrest on suspicion of driving under the influence of drugs (DUID). Research also shows that following GHB ingestion, subjects would still drive. Evaluation of the short-term stability of GHB is important to better understand and interpret GHB concentrations measured in oral fluid.

Objective: The objectives of the study were to develop and validate a gas chromatography-mass spectrometry (GC/MS) method for the quantitative determination of GHB in oral fluid, and to evaluate the short-term stability of GHB in oral fluid. The short time frame was selected based on the expected turn-around time of routine working laboratories from time of collection, to the reporting of results.

Method: Analysis for GHB was carried out using deuterated GHB as internal standard (IS; 100 µL at 10 mg/L), over a calibration range of 0.01 – 50 mg/L. The analytical method was adapted and validated for oral fluid from an in-house method optimised for the analysis of GHB in blood and urine.
Drug-free oral fluid was collected from a female volunteer and was frozen prior to the analysis. Oral fluid samples were spiked with GHB at 3 concentrations, 0.42, 4.2 and 42 mg/L. The IS was added prior to extraction. Acetonitrile was used to facilitate protein precipitation, following which the samples were centrifuged at 3000 rpm for 15 minutes. The acetonitrile was removed and evaporated to dryness at 40°C under nitrogen. Derivatisation with 75 µL of BSTFA + 1% TMCS was carried out prior to analysis using an Agilent 5975C/7890A GC/MS system with a DB5 column.
The method was validated in accordance with SWGTOX guidelines. The stability of GHB in oral fluid was assessed at three different temperatures; room temperature (24°C), refrigeration (4°C) and frozen (-22°C). Samples were analysed in duplicate and injected in duplicate.

Result: The method was successfully validated according to recommendations set in the SWGTOX guidelines, including linearity, specificity, sensitivity, carryover, bias, precision and accuracy.
Calibration graphs were linear, with an R2 value of 0.999. %CV values were less than 10% for all samples, excluding 3 samples at 24°C which exceeded 10%. The limit of detection (LOD) was determined to be 0.05 mg/L, the limit of quantitation (LOQ) as 1.0 mg/L.
GHB was found to be stable for up to 7 days at the three temperatures: concentrations ranged from 84% - 113% of the Day 0 concentration at 42 mg/L and 4.2 mg/L when stored in the freezer, 89% - 104% at fridge temperatures and 88% - 115% at room temperature. The low concentration could not be reliably quantified.

Conclusion/Discussion: A robust method was developed and validated for the analysis of GHB in oral fluid. Short-term, GHB was found to be stable over the testing period. Experimental and measurement error must be taken into account when assessing the results found, especially at low and endogenous concentrations in the 'blank', as these were too close to the limit of detection.

References: De Paoli G, Walker KM, Pounder DJ. Endogenous ɣ-Hydroxybutyric Acid Concentrations in Saliva Determined by Gas Chromatography Mass Spectrometry. Journal of Analytical Toxicology. 2011;35(3):148 -52.

Keywords: Gamma-Hydroxybutyrate (GHB), Oral Fluid, Stability

P14

Can a "Save Jar" Save the Day? Stability of Oxymorphone in Formalin Fixed Liver

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Introduction: At autopsy, specimens are collected for toxicology analyses, and preferably include central and peripheral blood, liver, urine, and vitreous humor. In addition to organ specimens sampled for microscopic examination, small segments of major organs are kept in formalin preserved "save jars." At the North Carolina Office of the Chief Medical Examiner, save jars are kept for a minimum of five years, whereas toxicology specimens are kept for two years. Two years after toxicology specimen disposal, the laboratory was contacted by an out of state law enforcement official. A 52 year old male's cause of death had been certified as natural means and manner with only a routine volatiles screening by the toxicology laboratory. In the following years the decedent's wife remarried several times, with each one of her husbands' dying a seemingly premature death. In these deaths there was a suspicion of poisoning by oxymorphone. Knowing that the first husband's specimens had been discarded, the laboratory was asked to conduct toxicology testing on whatever specimens remained. Enter the save jar.

Objective: The suitability of formalin fixed specimens for toxicology analysis was evaluated by screening save jar liver specimens, and their formalin solutions, for oxymorphone and comparing the results to frozen liver specimens stored in the toxicology laboratory.

Method: Four cases each from 2010 and 2012 were selected in which oxymorphone was detected during routine toxicological analysis and save jars were available for procuring formalin fixed liver specimens. The frozen liver specimens submitted to toxicology for analysis and storage had been discarded for the 2010 cases, but were available for the 2012 cases. Homogenates of each liver were prepared at a 1:4 dilution and analyzed by a targeted screen on a Thermo LXQ ion trap LC/MS. Confirmation was achieved with positive electrospray ionization using a Thermo TSQ triple quadrupole LC/MS/MS, collecting two MRM transitions for each analyte. Identification criteria are based upon retention time and ion ratios. Both screening and confirmation methods are fully validated.

Result: No oxymorphone was detected in formalin fixed livers, or their formalin solutions, in either the 2010 or 2012 cases. The toxicology laboratory's frozen liver from the 2012 cases all contained oxymorphone. In the analysis of the formalin solutions, oxymorphone was not detected, but of more interest, no oxymorphone-d3 internal standard was recovered, indicating that oxymorphone is not stable in formalin. The stability of oxymorphone and other drugs present in the targeted screen will be discussed, as well as a proposed mechanism for the reaction between oxymorphone and formalin.

Conclusion: Save jars can be a valuable resource when all other toxicology specimens have been exhausted or discarded, but it is important to know which analytes are stable in formalin. Oxymorphone, a potent opioid present in a large number of postmortem cases, is not stable in formalin, regardless of the specimen type. This evidence is important for the distinction between oxymophone "none detected" and "specimen unsuitable for analysis."

Keywords: Oxymorphone, Formalin, Postmortem

P15

Updates from the Drug Enforcement Administration National Forensic Laboratory Information System (NFLIS): 2014 Midyear Report

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Introduction: The National Forensic Laboratory Information System (NFLIS) is a program of the Drug Enforcement Administration, Office of Diversion Control that collects drug identification results from cases analyzed by Federal, State, and local laboratories. The system currently includes data from laboratories that conduct analyses of more than 91% of the nation's approximate one million annual State and Local drug cases. A total of 278 individual laboratories from State systems and local or municipal laboratories/laboratory systems participate in NFLIS. Results from NFLIS are regularly used to support drug scheduling efforts and to aid drug initiatives including the identification and tracking of emerging drugs of abuse.

Objective: The objective of this presentation is to provide the community with an overview of NFLIS data reported in the 2014 Midyear report and highlight two NFLIS resources, the Data Query System (DQS), and the Drug Information System.

Method: The 2014 Midyear report provides an overview of results of drug cases submitted to Federal, State, and local laboratories from January 1, 2014, through June 30, 2014, that were analyzed by September 30, 2014. This presentation provides highlights from the report including national and regional estimates for the top 25 identified drugs, national prescription drug trends, and the most common reported drugs in select drug categories including synthetic cannabinoids and phenethylamines. The DQS is an interactive tool that provides NFLIS participating laboratories with the ability to analyze NFLIS data at the national, regional, State, or local level. The DIS is a secure, moderated forum for the information sharing among participating laboratories, the DEA, and NFLIS project staff.

Result: There were 495,836 drug cases submitted to State and local laboratories during the 2014 Midyear reporting period. Cannabis/THC, methamphetamine, cocaine, and heroin were the most frequently reported drugs, accounting for 70% of all drug reports. Results show regional distributions of selected synthetic cannabinoids such as XLR11, AB-FUBINACA, and AB-PINACA and phenethylamines such as methamphetamine, amphetamine, and methylone.

Conclusion: Attendees will gain an understanding of the breadth of information reported to NFLIS during the first half of 2014 and how NFLIS publically shares data that can benefit management decisions of crime laboratories.

Keywords: National Forensic Laboratory Information System, Drug Enforcement Agency, Drug Trends

P16

Disposition of the 5-HT$_{2A}$ Agonist Designer Hallucinogen, 2-(4-chloro-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine (25C-NBOMe), in the Mouse

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Introduction: Since 2012, a new class of "2C" designer drugs, dimethoxyphenyl-N-[(2-methoxyphenyl)methyl]ethanamine (NBOMe) derivatives, have become available on the illicit drug market. Nicknamed "N-Bombs", these drugs are designer hallucinogens. They are potent serotonin 5-HT2A receptor agonists. This receptor has been linked to memory, cognitive processes that are involved in affective disorders and the effects of hallucinogenic drugs such as LSD. There is little to no data concerning absorption, metabolism and elimination of any of these NBOMe derivatives in man or whole animals. As of November 15, 2013 three of the NBOMe derivatives were added to schedule I, including 2-(4-chloro-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine (25C-NBOMe). We determined the disposition in mice of 25C-NBOMe in blood and six tissues at three distinct time points post-injection of 2 mg/kg 25C-NBOMe.

Objective: To determine the disposition of the psychedelic designer drug, 25C-NBOMe at 30min, 1hr and 4hr after subcutaneously injecting mice with a 2 mg/kg dose.

Experimental Method: Three groups of six C57BL6 mice were subcutaneously injected with 25C-NBOMe at a dose of 2mg/kg or vehicle (ethanol:emulphor:saline in a ratio of 1:1:18) at 30min, 1hr and 4hr after exposure. The mice were sacrificed and blood and tissue specimens were collected.

Analytical Method: A seven point matched matrix calibration curve (1-1000 ng/mL or 4-4000pg/g) was prepared with each analytical analysis. 1:1 blood:water was used. Tissues were weighed, water was added to equal 0.5 g, and homogenized. Internal Standard (200 pg/mL or 800 mg/g 25I-NBOMe-d3) and 0.5 mL of 1:1 100 mmol phosphate buffer (pH 6):methanol were then added to each sample. Samples were then mixed using a vortex mixer for 30 sec and then centrifuged for 5 min at 3000 rpm. Specimens were transferred to Clean Screen FASt^{TM} extraction columns and rapidly aspirated (15 s) with nitrogen under 80 psi using an UCT Positive Pressure Manifold (Bristol, Pennsylvania) into 250µL auto-sampler vials. Vials were then capped and the samples were analyzed by a previously published high performance liquid chromatograph tandem mass spectrometry (HPLC/MS/MS) method (*Poklis JL et al. Biomed. Chromatog. 2013, 27:**1794-1800***).

Result: The mean ± standard deviation of the blood, brain, heart, kidney, liver, lung and spleen concentrations of 25C-NBOME after subcutaneously injected at 30min, 1hr and 4hr (SD) were:

25C-NBOMe	Blood (ng/mL)	Brain (ng/g)	Heart (ng/g)	Kidney (ng/g)	Liver (ng/g)	Lung (ng/g)	Spleen (ng/g)
30 minutes	90 ± 32	400 ± 120	820 ± 300	1400 ± 540	140 ± 170	4300 ± 2200	2800 ± 750
60 minutes	68 ± 42	430 ± 130	510 ± 142	1100 ± 270	130 ± 140	2100 ± 1300	2100 ± 700
4 hours	2 ± 2	9 ± 4	14 ± 5	34 ± 15	10 ± 5	64 ± 55	100 ± 54

No 25C-NBOMe was detected in the vehicle injected groups of mice.

Conclusion: In general, blood and tissue concentrations of 25C-NBOMe after subcutaneous injection displayed a significant drop in concentration from 60 minutes to 4 hours which suggests rapid and extensive metabolism and excretion. If the blood concentration at either 30 or 60 minutes is at peak or a post-peak value, then the blood half-life of 25C-NBOMe in the mouse is approximately 30 to 45 minutes. A low concentration of 25C-NBOMe in blood compared to the higher concentrations in all the tissues indicates a high apparent volume of distribution.

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Keywords: NBOMe Derivatives, 25C-NBOMe, Distribution

P17

A Poly-Drug Overdose Fatality Involving Topiramate

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Introduction: Topiramate (Topamax®, Trokendi®) is used alone or with other medications to treat certain types of seizures. It is an anticonvulsant drug with CNS depressant effects and has a half-life of 19-23 hours. Topiramate is also being used to treat psychiatric disorders, migraine headache and neuropathic pain. Common adverse effects include somnolence, dizziness, impaired memory and concentration, nausea and diarrhea. Signs of overdose may include convulsions, drowsiness, poor coordination, slurred speech, confusion and agitation. Lamotrigine (Lamictal®) is use in the treatment of epilepsy, usually in combination with other anticonvulsant drugs. It has a half-life of 12-62 hours. Some adverse reactions associated with lamotrigine use include skin rash, dizziness, headache, somnolence, ataxia, blurred vision and vomiting. Lamotrigine is extensively metabolized, mainly by conjugation with glucuronic acid.

Objective: This poster presents the details of an acute overdose fatality involving topiramate in combination with other drugs.

Method: Suitable peripheral blood was not available for testing. Heart blood was screened for volatiles by headspace GC, and heart blood was used for drug screening by Enzyme Multiplied Immunoassay Technique (EMIT) and liquid-liquid alkaline extraction followed by GC/MS. Benzodiazepines were confirmed in-house using HPLC. All other quantitations (topiramate, trazodone, lamotrigine) were performed on heart blood by an external laboratory.

Result: The subject was a 52-year old white female who was found unresponsive in her residence by her duplex manager after a neighbor grew concerned for her and her domestic partner. EMS was called and death was confirmed on scene. The subject's medical history was significant for bipolar disorder, borderline personality disorder, previous suicidal ideation, peripheral neuropathy, and chronic back pain. The subject and her domestic partner were facing eviction, had their car repossessed and had recently gave their dogs away. Suicide notes were found in the residence, one written by each woman. The initial drug screen was positive for alprazolam, topiramate, lamotrigine, trazodone, and chlorophenylpiperazine. Heart blood was positive for ethanol at 20 mg/dL but vitreous tested negative. Benzodiazepines confirmation confirmed the presence of alprazolam at 344 ng/mL. Topiramate confirmed at 99,000 ng/mL, lamotrigine at 210,000 ng/mL and trazodone at 22,000 ng/mL.

Conclusion/Discussion: Topiramate, alprazolam, trazodone, and lamotrigine all confirmed above or very close to their critical values. According to Baselt, topiramate does not exhibit significant postmortem redistribution; heart/peripheral blood concentration ratios averaged 1.0. Also, most postmortem blood topiramate levels above 49,000 ng/mL resulted in deaths. This subject had a topiramate level of 99,000 ng/mL. In fatality cases reported in Baselt, the highest lamotrigine level reported in heart blood is 52,000 ng/mL. The lamotrigine level of 210,000 ng/mL in this case is much higher. The cause of death in this case was topiramate, alprazolam, trazodone, and lamotrigine overdose, and the manner of death was suicide.

Keywords: Topiramate, Trazodone, Lamotrigine, Overdose, Death Investigation

P18

Diphenhydramine Concentration in Urine and Hair Following Oral Administrations of Single Daily Doses

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Background: Diphenhydramine, primarily used for treating allergy and common cold symptoms, is an over-the-counter first generation antihistamine that has abuse potential. Although a few diphenhydramine abuse, overdose, and drug-facilitated assault cases have been reported, data for the drug concentrations in urine and hair from innocuous use are limited. Typical dosages are 25 or 50 mg daily.

Objective: We measured the drug concentrations in both urine and hair samples voluntarily provided by 3 male subjects who had taken diphenhydramine for innocuous use with self-reported use history.

Method: Approximately 20 mg of 1.5-inch hair segment proximal to scalp was aliquoted for acetone wash and pulverization. The powder was spiked with diphenhydramine-d_3 (internal standard) and incubated overnight in pH 10.2 buffer at 53°C, followed by liquid-liquid extraction with 9:1 hexane:isopropanol. The evaporated hair extracts were reconstituted with mobile phase. Urine samples were aliquoted at 1.0 mL, spiked with internal standard, and diluted with mobile phase at 1:10. The diluted urine was filtered through Clean Screen FASt™ SPE columns. Both prepared hair and urine samples were analyzed by liquid chromatography-tandem mass spectrometry (3200 Sciex triple-quadrupole mass spectrometer) equipped with 50x2 mm, 2 μm Synergi Hydro RP column (Phenomenex). Three male volunteers took 25 or 50 mg single dose of diphenhydramine daily for different periods of time. The times of drug intake, as well as hair and random urine sample collections, were recorded. Both measured (ng/mL) and creatinine content-normalized (ng/mg creatinine) concentrations in urine samples were reported.

Result: The assays were validated following SWGTOX guidelines. The assays linear ranges were 10–3000 ng/mL for urine, and 40–2000 pg/mg for hair. Diphenhydramine concentrations in urine were insignificantly affected by three freeze-thaw cycles. Male #1 who took four consecutive 25 mg daily doses had diphenhydramine 111 – 872 ng/mL (317 – 687 ng/mg creatinine) in the urine samples collected within 3 – 12 hours after each dose. The urine samples collected approximately 14 – 24 hours after each dose (0 – 10 hours prior to each next dose) decreased to 60 – 341 ng/mL (106 – 274 ng/mg creatinine). Drug concentration dropped below lower limit of quantitation 10 ng/mL after 4 days of the last dose. The first hair sample of Male #1 was collected 12 hours after the first 25 mg dose, and the drug concentration was 87.9 pg/mg. Additional hair samples were collected at 4, 11, 18, 25, 53, and 60 days after Male #1's last 25 mg consecutive daily dose. Sequential diphenhydramine concentrations of these hair samples were 37, 132, 37, 69, 143, and 146 pg/mg. Male #2 took 50 mg daily dose for 2 consecutive days. Diphenhydramine concentration in hair sample collected 18 days after his last dose was 112 pg/mg. Male #3 had diphenhydramine concentration in hair at 372 pg/mg after 50 mg daily dose for the past 2 weeks.

Discussion/Conclusion: We reported diphenhydramine concentrations in urine and hair samples of 3 male subjects. With limited sample size and different use history provided, diphenhydramine appeared in urine at concentrations lower than 1000 ng/mL (less than 700 ng/mg creatinine), and lower than 400 pg/mg in hair. The drug can be detected in urine within 4 days after, but in hair for at least 8 weeks after last oral administration. When dealing with toxicology cases involving diphenhydramine, the concentrations must be interpreted with caution.

Keywords: Diphenhydramine, Hair and Urine Analysis, Innocuous Oral Administration

P19 ***"NIJ Funded"***

Simultaneous Identification of Twenty-Two Synthetic Cathinones in Urine Using LC/Q-TOF-MS

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Introduction: The ongoing proliferation of designer drugs present a variety of public health and public safety concerns. Synthetic cathinones are capable of producing a variety of psychostimulant effects and according to the National Forensic Laboratory Information System (NFLIS), their use has escalated considerably. There have been numerous published reports involving synthetic cathinones in antemortem and postmortem toxicology investigations. Due to limitations in immunoassay-based screening technologies, many forensic toxicology laboratories must rely on more labor intensive chromatographic-based screening approaches in order to detect these drugs in biological evidence.

Objective: To develop and validate an analytical procedure for the determination of twenty-two synthetic cathinones in urine using liquid chromatography/quadrupole-time of flight-mass spectrometry (LC/Q-TOF-MS).

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: methcathinone, ethcathinone, pentedrone, buphedrone, 3-fluoromethcathinone (3-FMC), 4-fluoromethcathinone (flephedrone, 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), α-pyrrolidinopentiphenone (α-PVP), pyrovalerone, and naphyrone. A total of nine deuterated internal standards were employed (methylone-d3, eutylone-d5, pentylone-d3, butylone-d3, MDPV-d8, naphyrone-d5, mephedrone-d3, α-PVP-d8, and ethylone-d3). A targeted analysis was performed using a minimum of two transitions from each precursor ion. Unlike other published methods, water losses were not permitted. Fragments were structurally identified and transitions were selected to enhance overall specificity. The procedure was validated in accordance with the Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation. The parameters assessed included analytical recovery, calibration model, carryover, bias, precision, limit of detection, limit of quantitation, matrix effect, interferences and dilution integrity.

Result: Solid phase extraction yielded extraction efficiencies in the range 84-104%. Quantitative analysis was achieved using a linear, unweighted calibration model from 0 to 1,000 ng/mL. Residual plots and correlation coefficients were used for the evaluation. Limits of detection (LOD) and quantitation (LOQ) were determined using three sources of matrix in duplicate over three runs. LODs ranged from 0.25-5 ng/mL and LOQs ranged from 0.25-10 ng/mL. Bias and precision were assessed using pooled fortified matrix at 10, 100, and 800 ng/mL in triplicate over five runs. Bias for all twenty-two analytes ranged from -1-12%, -3-4%, and 1-8% for 10, 100 and 800 ng/mL, respectively. Inter-assay and intra-assay precision were assessed. Inter-assay precision ranged from 4-12%, 2-12%, and 3-9% for 10, 100, and 800 ng/mL, respectively. Intra-assay precision ranged from 1-11%, 0-7%, and 0-8% for 10, 100, and 800 ng/mL, respectively. Ion suppression and enhancement for each analyte and internal standard were evaluated at 20 ng/mL (-22% to -1%) and 200 ng/mL (-21% to -2%) using ten independent sources. Interferences were evaluated in terms of matrix (ten independent sources), contributions from isotopically labeled internal standards, common drugs, and structurally related compounds. In addition to the common drugs, more than twenty-five amphetamines and amphetamine-like designer drugs were included in the interference study. Qualitative interferences were evaluated using negative and positive controls fortified with target analyte (10 ng/mL and 100 ng/mL) in the presence of interferent at a 10- or 100-fold increased concentration (1000 ng/mL). Carryover was evaluated at 1000, 2500 and 5000 ng/mL. No carryover was observed with the exception of naphyrone at 5000 ng/mL. Finally, dilution integrity was verified using two and four-fold dilutions, yielding accuracies within ±20% of the expected value.

Conclusion: LC/Q-TOF-MS was used to identify twenty-two synthetic cathinones in urine following solid phase extraction. The method was validated in accordance with the SWGTOX Standard Practice for Method Validation document. This new procedure was developed as part of a larger study to systematically evaluate the stability of synthetic cathinones in biological evidence.

Keywords: Cathinones, Designer Drugs, Validation, LC/Q-TOF-MS

P20

Detection of 68 Drugs and Metabolites in Umbilical Cord Tissue: Comparison of Validated Methods: LC-MS/MS to LC-TOF-MS

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Introduction: Umbilical cord tissue, as an alternative to meconium, provides easier collection and a larger volume of specimen for the assessment of pre-natal drug exposure. Currently, this detection is performed using liquid chromatography time-of-flight (LC-TOF) mass spectrometry. However, the LC-TOF platform has limitations, including specificity of isobaric pairs, which prevents using faster chromatographic methods to increase throughput. In addition, two separate injections are required on the LC-TOF to collect positive and negative ion data.

Objective: 1)To compare the current LC-TOF method to a newly developed LC-MS/MS method for the analysis of 68 compounds in umbilical cord. 2)To implement a single point calibration standard for each compound for qualitative reporting and statistical quality control (QC) monitoring.

Method: One gram of umbilical cord tissue was suspended in 2 mL of DNase and internal standard solution and homogenized. After centrifugation, 1mL of supernatant was transferred to a Biotage SLE+ plate. Extracts were evaporated and reconstituted in starting mobile phase.

Samples were analyzed using both an Agilent 6230 TOF Mass Spectrometer and an Agilent 6460 Triple Quadrupole Mass Spectrometer, each with Agilent 1260 Liquid Chromatography systems.

The newly validated LC-MS/MS chromatographic method used a Phenomenex Phenyl-Hexyl 50x3, 2.6µm column at 55°C with an aqueous mobile phase containing 5mM ammonium formate with 0.1% formic acid and an organic mobile phase of 80:20 methanol:acentonitrile with 0.1% formic acid. A gradient from 5% organic to 98% organic over 3.5 minutes provided separation of all compounds. 15µL of sample was injected at a flow rate of 0.5 mL/min. The MS/MS was operated in dynamic MRM mode with 156 transitions (68 compounds, 10 internal standards, 2 transitions each). The umbilical cord LC-TOF method was conducted as previously published.[1]

Result: The analysis used in the LC-MS/MS method incorporated polarity switching resulting in a 5 minute cycle time, compared to the TOF's two injections and a combined ~11.5 minute cycle time, resulting in a 55% time reduction. A single point calibration standard at each cutoff with regression through zero, a 50% (negative) control, a 150% (positive) control and a true negative were included with each batch. Results were reported qualitatively; however, quantitative data was used for statistical QC analysis and for laboratory interpretation. All compounds were referenced to one of ten internal standards. The ten internal standards were found to be acceptable for qualitative result reporting based upon method comparison with an outside laboratory and imprecision studies.

514 patient samples were run by both methods over 20 days. Of the 260 LC-TOF negative samples, 259 were LC-MS/MS negative. 516 individual compounds from the LC-TOF positive samples were identified using the LC-MS/MS; however 55% were below the established qualitative cutoff. In addition, nine additional compounds were found on the LC-MS/MS that were not identified with the LC-TOF method due to unacceptable match scores in the TOF database.

Inter-assay imprecision conducted over 20 days using the 50% and 150% quality control samples yielded an average % deviation from the target of 112% and 121%, respectively. No carryover was observed with a sample at 150% of the cutoff. Ion suppression was evaluated by post-column infusion of the compounds of interest. No ion suppression was observed.

Conclusion: In comparison to the existing LC-TOF method, the LC-MS/MS method offers a single injection solution with faster chromatography, resulting in an increase in laboratory throughput while maintaining a high level of specificity. Use of a single-point calibration standard and ten representative internal standards provided enhanced accuracy around the established cutoff and the use of statistical QC for routine monitoring.

1. Marin SJ, et al. *Ther Drug Monit.* 2014 Feb;36(1):119-24

Keywords: Umbilical Cord Tissue, Drugs of Abuse, LC-MS/MS

P21
Differences in Drugs of Abuse Detected Among Twins at Birth Using Umbilical Cord: A Case Study

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Background/Introduction: Twins can be monozygotic or dizygotic. Dizygotic twins will always have 2 placentas. Monozygotic twins can share a single placenta and an amniotic sac or a single placenta and 2 amniotic sacs 75% of time, while 25% of the time, will have separate placentas and separate amniotic sacs. A previous study reported that 0.9% of all meconium results for multiple births were inconsistent and 4% of cocaine results for twins were inconsistent. Umbilical cord results for twins are lacking in the literature.

Objective: The objective to report a case where the umbilical cord benzoylecgonine results for a pair of identical twins were very different while the hydromorphone results were similar.

Method: Our laboratory received segments of umbilical cord that originated from a pair of monozygotic twins for routine toxicology analysis. For screening analysis, umbilical cord was aliquoted and homogenized in 3 mL acetone. After centrifugation and filtration, the filtrate was added with 50 □L of 0.2% acetonic succinic acid to be evaporated, and was reconstituted in provided buffer subjecting to opiates and cocaine heterogeneous-competitive enzyme-linked immunosorbent assays (ELISAs). Both ELISAs were calibrated at 0.5 ng/g cut-off. The absorbance readouts of the samples were normalized by negative controls (B/B_0), and compared with those of the calibrators.
For confirmatory testing, a separate umbilical cord (0.5g) aliquot is added with internal standard (Hydromorphone-d_3 and Benzoylecgonine- d_8)and homogenized in 3 mL of acetonitrile. Following centrifugation, the supernatants were decanted, evaporated, and reconstituted in 3 mL phosphate buffer (pH 6), subjecting to solid phase extraction. The final extracts were analyzed using validated liquid chromatography tandem mass spectrometry (LC-MS/MS). The lower limit of quantitation (LLOQ) was 0.2 ng/g and the limit of detection (LOD) was 0.1 ng/g for both hydromorphone and benzoylecgonine.

Result: The B/B_0 from the opiate ELISA for calibrator, baby #1, and baby #2 were 65.7%, 69.4% (negative), and 64.6% (positive), respectively. In other words, both samples were near the cut-off. On the other hand, the B/B_0 from the benzoylecgonine ELISA for calibrator, 0.25 ng/g control, baby #1, and baby #2 were 68.5%, 76.9%,77.1% (near 0.25 ng/g), and 6.9%, respectively. Baby #1 was thus reported with negative results, and Baby #2 was reflexed for opiates and benzoylecgonine LC-MS/MS confirmatory test, leading to hydromorphone (0.95ng/g) and benzoylecgonine (6.0 ng/g) positive results. Due to the apparent discrepant results, the client requested that both specimens be retested by LC-MS/MS. The retest results for baby #1 were hydromorphone (1.06 ng/g) and benzoylecgonine (0.31 ng/g) and baby #2 hydromorphone (0.81ng/g) and benzoylecgonine (5.41 ng/g).

Conclusion: The twins in our study were monozygotic twin who shared a placenta but had two amniotic sacs. The hydromorphone results were very similar. However, the benzoylecgonine results were very different with baby #1 being approximately half of the cutoff and baby #2 being significantly elevated from the cutoff. Maternal administration of *Dilaudid during labor may be explanatory for the similar hydromorphone results. However,* there were significant differences in the concentration of benzoylecgonine between the twins where it may be assumed that usage occurred over a longer period of time. In Twin-to-twin transfusion syndrome (TTTS), one fetus receives more blood than the other, which could be a possible explanation for difference in detection of benzoylecgonine between the twin babies in our study. Retrospective studies of multiple births need to be undertaken to better understand this observation.

Keywords: Hydromorphone, Benzoylecgonine, Monozygotic Twins, Umbilical Cord, Tandem Mass Spectrometry

P22

35 Drugs and Metabolites by LC-TOF-MS: Doubling Throughput without Compromising Specificity

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Background: Compliance testing is a rapidly growing portion of the drug testing market. In many cases, patients are prescribed drugs with high street value and high potential for addiction and abuse. Because of this, when patients enter pain management programs, compliance testing is performed to ensure they are taking the medication as prescribed. Due to differences in urine potency, quantitation is largely uninformative in urine testing. As an alternative to conventional immunoassay screens with reflex to confirmation, we previously published a mass spectrometry-based screen for compliance testing in urine using liquid chromatography time-of-flight mass spectrometry (LC-TOF)[1]. The original validated method required 20 hours of instrument time for a complete batch. With continued optimization of key quality parameters to aid in result interpretation, a faster chromatographic method was investigated.

Objective: The objectives were 1) to increase the throughput of the current clinically validated assay by shortening the chromatographic method, 2) ensure qualitative agreement between the current and modified methods, and 3) resolve identified interferences chromatographically to improve specificity of the method.

Method: Briefly, the cutoff calibrator, controls at 50% and 150% of the cutoff in matrix, and patient specimens were extracted using Biotage Isolute SLE+ 96-well (Charlotte, NC, USA) plates by adding 300 µL of urine mixed with internal standard-containing bicarbonate buffer. A positive pressure manifold was used to push sample into the packing material and allowed to absorb for 5 minutes. Samples were eluted with ethyl acetate and dried under nitrogen at 40^0C for 10 minutes. They were resuspended in a 90:10 mix of the mobile phases. Samples were analyzed using an Agilent 1260 Rapid Resolution HPLC coupled to an Agilent 6230 accurate mass time-of-flight mass spectrometer (Santa Clara, CA, USA). Chromatographic separation was achieved on a Phenomenex 2.1x50 mm Kinetex F5 column (Torrance, CA, USA). Mobile phases were 5mM ammonium formate in water, pH 3.5, and Optima grade methanol (Thermo Fisher Scientific). Using a 1 mL/min flow rate, mobile phase B was ramped from 10% to 95% over 3 minutes. Data were analyzed using the Agilent Mass Hunter Quantitative Analysis software. A one point calibration forced through the origin was used to determine positivity, along with mass accuracy, retention time matching, and isotope abundance/spacing. Controls fortified at 50% and 150% of the cutoff were used to evaluate accuracy and reproducibility within each batch.

Result: Twenty batches containing patient specimens previously analyzed with our current, clinically-validated method were re-analyzed using the improved chromatography as part of a method comparison. All results showed excellent correlation with the original method, suggesting previously validated parameters (matrix effects, imprecision, recovery, detection thresholds, etc.) were not compromised. Within-run retention time stability was evaluated by re-injecting controls at the end of the batch. No observable retention time shifts were noted after approximately 1500 injections. The new chromatographic method reduced the overall analysis time by 55% per injection with no qualitative impact on results.

Conclusion/Discussion: Throughput was doubled without compromising the specificity of the assay. Although fragmentation may resolve some isobaric pairs, there remain numerous drugs of interest where chromatographic separation is needed for accurate identification. In the presented assay, six isobaric compound pairs and known interferences were all resolved through chromatographic separation, mass accuracy, and/or isotopic spacing using this new method.

1. McMillin, G. A., et al. (2015). "A hybrid approach to urine drug testing using high-resolution mass spectrometry and select immunoassays." Am J Clin Pathol **143**(2): 234-240.

Keywords: High Resolution Time-of-Flight Mass Spectrometry, Compliance, One-Point Calibration

P23

Fast, Simple, and Accurate Method for Urine Drugs of Abuse Screening and Quantitation Using Liquid Chromatography with Time of Flight (TOF) Mass Spectrometry

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Background/Introduction: Sample preparation is costly and time consuming. Methods with minimal preparation that produce clean chromatography and accurate and reproducible measurement by Time of Flight (TOF) mass spectrometry are highly desirable for high throughput clinical and forensic laboratories.

Objective: We describe here a validated method with minimal sample preparation of urine and very clean chromatography with accurate and precise analysis using a LC –TOF-MS.

Method: Fifty microliters of urine, 25 uL β-glucuronidase (IMCSzyme), 50 uL acetate buffer (pH = 7), and 50 uL of working internal standard solution were added to a 96 well plate which was vortexed and incubated (hydrolyzed) in a heating unit for 1 hour @ 60 ºC; 50uL of mobile phase A (see below) was then added and centrifuged at 3000 RPM for 5 min. which mixed the sample and removed any particulates. Two hundred microliters were aspirated into a 96 well analytical plate. Chromatographic separation of the analytes was performed on a Shimadzu LC-20 AD Liquid Chromatograph system with Perkin Elmer analytical column (Brownlee SPP 2.7 *u*m: C18: 2.1 x 100mm) @ 40ºC and a flow rate of 0.4 mL/min. Injection volume was 5 uL. A linear gradient was run with 0.1% formic acid in water (Mobile phase A) and 0.1% formic acid in acetonitrile (Mobile Phase B). Initial conditions were 95% A / 5% B, and then a linear gradient to 70% A / 30% B from 1.5 to 3.5 min. with a second linear gradient to 10% A / 90% B from 3.5 to 9.5 min and returned to initial conditions for 3 min. The mass spectrometer was a Bruker Compact Quadrupole Time of Flight (QTOF) mass spectrometer (MS) operating in positive electrospray mode. The instrument was calibrated with sodium formate clusters at the beginning of every injection and mass accuracy of less than 2 ppm. Data were acquired using an acquisition rate of 3 Hz over the mass range of 50 – 1000 m/z. Quantitation was based on high resolution extracted ion chromatograms (5 mDa). All data were processed with TASQ 1.0 software. Calibrator levels to determine LOQ and ULOL were prepared at values from 5 – 500 ng/mL for the drugs/ metabolites expected at lower concentration or 50-5000 ng/mL for drugs/metabolites expected at higher concentrations.

Result: The following drugs/metabolites were analyzed: Opiates/Opioids: morphine, codeine, hydrocodone, hydromorphone, oxycodone, oxymorphone, meperidine, buprenorphine, methadone, methadone metabolite (EDDP), mono-acetylmorphine, fentanyl, norfentanyl; Benzodiazepines: alprazolam, alphahydroxyalprazolam, diazepam, nordiazepam, oxazepam, temazepam, lorazepam, clonazepam, 7-aminoclonazepam; Stimulants: cocaine metabolite (benzoylecgonine), amphetamine, methamphetamine, MDMA, MDA; Tricyclic Antidepressants: amitriptyline, nortriptyline, imipramine, desipramine, doxepin and others: tapentadol, tramadol, carisoprodol , meprobamate, zolpidem, PCP

A calibration curve (performed daily) of peak area ratio with each drug or metabolite's respective deuterated internal standard vs target concentration was linear with a greater than 0.99 correlation coefficient. LOQ and ULOL were determined from replicates of 5 for each concentration at $\pm$ 20% of the target concentration. Benzodiazepines CV% at LOQ and cutoff averaged 3.6% and 2.6%, respectively. Opiates/Opiods CV% at LOQ and cutoff averaged 2.6 % and 3.7 %, respectively and all other drugs listed above averaged 4.3% CV% at LOQ and cutoff. No interferences were found and carryover defined as ULOL.

Conclusion/Discussion: This method with minimal sample preparation and use of a purified form of β-glucuronidase, combined with high resolution, accurate mass analysis using a QTOF mass spectrometer produced excellent and reliable results. This method could easily be adopted in any clinical or urine drugs of abuse screening laboratory.

Keywords: Drugs of Abuse, LC-MS, TOF

P24 ***"NIJ Funded"***

Designer Drug Portable Database and Spectral Library

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Background: Mass spectral databases are of high relevance for forensic practitioners and anyone involved in the detection and identification of novel psychoactive substances ("designer drugs") in biological specimens. The generation of such databases is not an easy task, since it requires sound experimental design for method development, method optimization, method validation and interpretation of the data that is being generated. The traditional way of sharing spectral data is via databases, some of which are commercially available (*e.g.,* the Wiley designer drug database). Others are available in free access pages like SWGDRUG, Forensic DB.org, Cayman Chemicals, Forendex, and Designer Drugs Online (German database with more than 2000 entries). Each one of these databases offers information on individual mass spectra of relevant designer drugs and analogs. However, the inclusion of spectral data in public and commercial databases from different sources (*e.g.,* street drug or neat standards) may represent a problem when conducting match comparisons between the actual case and the database.

Objective: The purpose of this project was to create a portable designer drug database web application that could be easily accessed by forensic practitioners with the intention of visualizing relevant mass spectral information presented in a tabular format. This project consists of a convenient way for researchers at Florida International University (FIU) to share a comprehensive mass spectral numeric database for designer drugs for other LC-MS users.

Method: The FIU-Designer Drug Portable Database Library is being built into a single page web application using a model view controller (MVC model) by using some of the latest software technologies on the market. For example, it uses AngularJS as a structural framework for dynamic web apps developed by Google. This application also uses Bootstrap, which is the most popular HTML, CSS, and JavaScript framework for developing responsive web design applications. Lastly it uses PHP for server side programming, HTML, Javascript and css for client side programming.

Result: The different web tools used facilitated the creation of the web application for the easy sharing of spectral data obtained from LC-QqQ-MS and LC-QTOF-MS instrumentation. The application incorporates spectral information given as numeric values and ratios of the most important precursor and product ions generated during positive electrospray ionization work in an LC/MS instrument. Chemical formula, chemical structure, CAS number, and ChemSpider number are also included as part of the basic information for the user. Single unit resolution as well as accurate mass were incorporated into the database. A link to the web application is http://designerdrugs-dev.cs.fiu.edu/#/home. The user can type in JWH-398 as an example.

Conclusion: The final users of this application will benefit from having access to a dedicated spectral library database for designer drugs.

Keywords: Designer Drugs, Database, Web Application

P25

Ultra-Fast Targeted Screening of 35 Drugs of Abuse in Urine at 9 Seconds Per Sample Using SALLE Extraction and Laser Diode Thermal Desorption Mass Spectrometry (LDTD-MS/MS)

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Background/Introduction: Several immunoassays were used to screen different drug families in urine samples. To reduce the number of screening assays, laboratories moved to mass spectrometry detections. The screened drugs have different polarities and need a long LC chromatographic analysis to differentiate these different families. To increase the sample analysis throughput, Laser Diode Thermal Desorption Mass Spectrometry (LDTD-MS/MS) using fast ß-glucuronide digest and generic Salt Assisted Liquid-Liquid Extraction (SALLE) is evaluated.

Objective: LDTD-MS/MS offers specificity combined with an ultra-fast analysis for an unrivaled screening method. To develop this application, we focused on performing fast and simple extraction methods using a SALLE procedure. Thirty-five drugs of abuse from different classes (opioids, benzodiazepines, amphetamines, barbiturates, cocaine, PCP, 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 cutoff concentrations suggested by SAMSHA or those reported by Toxicology laboratories.[1] The following drugs were screened using positive ion mode: nordiazepam, 7-aminoflunitrazepam, diazepam, 7-aminoclonazepam, oxazepam, estazolam, temazepam, alprazolam, lorazepam, α-OH-alprazolam, 2-OH-ethylflurazepam, α-OH-midazolam, α-OH-triazolam, chlordiazepoxide, clonazepam, flunitrazepam, amphetamine, methamphetamine, BZE, cotinine, dextromethorphan, MDMA, MDEA, MDA, methadone, EDDP, codeine, morphine, oxycodone and PCP. The following drugs were screened using negative ion mode: butalbital, secobarbital, phenobarbital, amobartital/pentobarbital and butabarbital. Samples were extracted using a generic sample preparation, SALLE. Ten microliters of urine samples were mixed with 3 µL of purified ß-glucuronide (IMCSzyme) and 4 µL of internal standard solution diluted in a rapid hydrolysis buffer. A fast digestion was performed at 55°C for 15 minutes; 75 µL NaCl (saturated solution in water) and 300 µL of acetonitrile were added. After sample mixing by vortex and phase separation, 2 µL of upper layer are spotted in a LazWell pre-coated plate with EDTA. Analysis is performed in LDTD-MS/MS after evaporation to dryness.

Result: The LDTD-MS/MS was operated in MRM mode to provide rapid measurement of all drugs that desorbed simultaneously. Specific transitions were monitored for each drug to quantitate calibrator levels. Ionization was performed in positive mode for group one and negative mode for the second group. Analysis included spiked drugs in urine, potentially interfering drugs and real samples. All compounds gave good linear responses around the cutoff concentration. MRM transition specificity was evaluated by monitoring all transitions while desorbing individual drugs spiked at 1000 ng/mL. Using this shotgun approach, no specificity is distinguished between the codeine /hydrocodone transitions, morphine/hydromorphone transitions and the amobarbital/pentobarbital transitions due to similar elemental composition and fragmentation patterns. This limitation is an expected characteristic of a targeted screening approach where only positive or negative outcome is expected. Drug concentrations from 38 real samples were also evaluated using LC-MS/MS method with a long gradient to separate each drug class. For each drug in real samples, correlation of the data generated by LDTD-MS/MS and LC-MS/MS was evaluated. No false positives are observed except for 7-aminoclonazepam and flunitrazepam where 9.5% of all tested samples yielded a false positive. No false negative were obtained but a β-glucuronidase treatment is essential to detect the free drug form.

Conclusion/Discussion: The LDTD technology combined with generic sample preparation (SALLE) allows for robust drug screening in urine samples with quantitation levels close to the cutoff level recommended by SAMSHA. Sample-to-sample run time of 9 seconds is achieved with the capability to simultaneously analyze 30 drugs in positive MRM mode and 5 drugs in negative MRM mode.

Reference: 1) Redwood Lab and Quest website drug screening information

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

P26 ***"NIJ Funded"***

The Risk of Electronic Cigarettes to Public Health and Criminal Justice

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Background/Introduction: The Food and Drug Administration (FDA) has currently not developed regulations on the distribution of electronic cigarettes for non-therapeutic use. Conceivably this allows children, not old enough to buy traditional cigarettes, to purchase electronic cigarettes at their neighborhood "vape store". Vape shops are increasing in number and commercials tout these devices as a new way to quit smoking. While the FDA debates a unifying policy, states and localities are passing legislation to tax electronic cigarettes, ban sales to minors, and ban vaping indoors or in public locations. One of the largest driving forces behind legislation in some states is the danger of nicotine overdoses in children.

Additionally, electronic cigarettes have become an undefined criminal justice problem. People are modifying and adulterating electronic cigarettes and e-liquids to "vape" a variety of illicit drugs in attempts to conceal using them. The variety and availability of electronic cigarettes is prolific, yet the overall criminal justice impact is widely unknown. Data mining is imperative to define the scope of the risk.

Objective: The goal was to identify information and resources to assist ongoing research to understand the forensic impact and implications of electronic cigarettes. Information gleaned in the process of this study will aid publications that could impact decisions regarding criminal justice and public education.

Method: Data was collected from Internet sources including Google Alerts utilizing the search terms: electronic cigarettes; nicotine electronic cigarettes; illegal drugs electronic cigarettes; legislation electronic cigarettes; electronic cigarette policy (by state); criminal justice electronic cigarettes; heroin electronic cigarettes; marijuana electronic cigarettes and methamphetamine electronic cigarettes.

Primary sources such as YouTube videos and drug blogs were collected and cataloged. Additionally, interviews were conducted with experienced users regarding usage patterns, device configurations and manipulations. Trending media reports were cataloged and policy data was compiled into a table representing state lawmaking trends.

Result: Modifications and adulterations to electronic cigarettes and e-liquids, including illicit drug delivery, are described and promulgated by experienced users through videos, social media, and user blogs. All fifty states have developed policy in at least one of these five areas: sales to minors, defining electronic cigarettes as tobacco products, bans on use in public, taxes or other regulations. Numerous websites and blogs (ie. Reddit, YouTube) describe using electronic cigarettes to deliver illicit drugs such as marijuana, methamphetamine, and heroin. Media has also reported deaths due to e-liquid consumption.

Conclusion/Discussion: The electronic cigarette industry is in a state of quandary. State legislation is a piecemeal effort to regulate the electronic cigarette industry while the federal government continues to solicit research, both scientific and policy-based, to develop unifying regulation.

As licit and illicit use of electronic cigarette devices proliferate, the risk to public health and criminal justice needs to be fully defined. This research will inform the forensic toxicology community of the problems and dangers of adulteration and manipulation of electronic cigarettes and e-liquids.

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Keywords: Electronic Cigarettes, Data Mining, Policy

P27

Applications for DART™ MS Technology for Bioanalysis of Opioids

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Background/Introduction: Biological specimen analysis in forensic toxicology laboratories is a time and labor intensive process, which in some instances creates case backlogs. Additionally, the 2009 National Academy of Science report cites lack of personnel and funding as contributing factors. These backlogs are further compounded by society's increased drug abuse. Implementation of newer technology and methodology such as Direct Analysis in Real Time mass spectrometry, DART™ MS, has the potential to alleviate such issues by reducing analysis time and dedicated resources.

In this study, DART™ MS technology was investigated as a platform for qualitative opioid analysis through a comparison of data collected from four sample-processing techniques that were performed on three biological specimens (whole blood, urine, and vitreous humor). DART™ MS instruments provide rapid results (2-3 minutes) by relying on mass spectrometers rather than chromatography for separation. Two DART™ instruments were selected for this study; the DART™ TOF MS was utilized for screening and the DART™ QTRAP™ MS provided potential confirmation. This evaluation was designed as a proof-of-concept study; therefore, potential confirmation issues caused by structural isomers will be addressed in future validation studies.

Objective: In this study, our laboratory presents an evaluation of Direct Analysis in Real Time mass spectrometry "DART™ MS" as an alternate opioid analysis platform for toxicological specimens through conventional and emerging simple sample-processing techniques.

Method: Due to the complexity of the biological specimens, sample-processing techniques are required. Here, four such techniques were compared using fortified biological media including whole blood, urine, and vitreous humor. Each of these media was spiked with six analytes representative of the opioid class including: tapentadol, methadone, morphine, 6-monoacetylmorphine, hydrocodone, and oxymorphone. Next, the media was processed using the following sample processing methods: protein precipitation (PPT), liquid-liquid extraction (LLE), solid-phase extraction (SPE), and dried blood spotting (DBS). To properly evaluate each sample-processing technique, multiple protocols were performed within each category. A series of four samples were prepared for each protocol and consisted of (a) a low/therapeutically relevant concentration, (b) a high/toxicologically relevant concentration, (c) a negative control (contained deuterium labeled analyte only), and (d) a matrix blank. All samples were prepared in 1mL of authentic human matrix and were analyzed by both DART™ MS instruments.

Result: Due to the DART™ TOF MS instrument's increased resolution, overall detection of the selected opioids was more comprehensive compared to that of the DART™ QTRAP™ MS. On the DART™ TOF MS, 5 out of 6 analytes were consistently detected across the four sample-processing techniques, which demonstrated it to be highly sensitive. In contrast, because of sensitivity, the DART™ QTRAP™ MS was not as effective in detecting the selected analytes, results ranged between 2 and 4 analytes out of 6. These data reveal the DART™ QTRAP™ MS required more thorough sample-processing techniques. Additionally, data from both instruments revealed that polar analytes, methadone and tapentadol, provided consistently higher responses than non-polar, classical structured opioids throughout all processing techniques.

Conclusion/Discussion: From these data, no one all-inclusive technique is possible for opioid bioanalysis due to diverse chemistries in opioid structures; however, these data effectively demonstrate that DART™ MS can be successfully applied for qualitative toxicological analysis. Therefore, we offer DART™ MS as a high throughput, cost effectiveness, and highly sensitive alternate platform for detection of opioid analytes from biological specimens.

Keywords: Direct Analysis in Real Time Triple Quadrupole Linear Ion Trap Mass Spectrometry (DART™ QTRAP™ MS), Direct Analysis in Real Time Time-of-Flight Mass Spectrometry (DART™ TOF MS), Bioanalysis

P28

Development of an LC/MS/MS Naloxone Hydrolysis Assay Using a Recombinant Beta-Glucuronidase for Urinalysis

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Objective: The development and validation of a hydrolysis assay for the detection of naloxone, nornaloxone and naloxol in urine.

Background: Naloxone is a synthetic narcotic that has been in clinical use for decades. Its principal function is as an opioid antagonist that counters the effect of opioid overdose. It is typically administered intravenously and works to reverse nervous system depression that results from toxic levels of opioids present in the bloodstream. Naloxone is also coformulated with buprenorphine (Suboxone®, Zubsolv®), which was approved for maintenance treatment of opioid dependence in 2002. Naloxone in this form serves to prevent misuse by producing withdrawal symptoms if this medication is taken otherwise than sublingually. Given the nature of this application, naloxone is targeted in medication monitoring programs for drug adherence. The chief urinary metabolites are reported to be conjugates of naloxone, nornaloxone (noroxymorphone) and naloxol and thus analysis should include a hydrolysis step.

Method: IMCSzyme™ recombinant beta-glucuronidase was investigated based on its proven hydrolysis efficiency abilities with our other lab assays for enzyme hydrolysis for the naloxone assay. The enzyme was combined with internal standard, naloxone-D5, and buffer at pH 7 to produce a mastermix solution. Urine samples were treated 1:10 with the mastermix. Hydrolysis efficiency was tested at 55° C and 65° at incubation times 0, 15, 30, 45 and 60 minutes with a 3000 ng/mL naloxone-3-glucuronide control. Hydrolyzed urine samples were analyzed without any further sample preparation on a Waters Acquity UPLC® TQD using a 2.1 minute chromatographic gradient. The method was set up to test for the presence of naloxone, nornaloxone and naloxol in positive electrospray ionization mode. Validation parameters included: limits, linearity, precision and accuracy, matrix effect, interference and patient evaluations. Suboxone® patient samples (n=36) were analyzed for the presence of naloxone and metabolites.

Result: The optimal amount of enzyme required for complete hydrolysis was determined to be 3000 Fishman units. However, the sucrose stabilizer of the enzyme required a 3X dilution for the preservation of column life. Optimal hydrolysis took place at 65°C at 60 minutes, but only provided 70.9% hydrolysis efficiency for the targeted 1952.6 ng/mL naloxone concentration with the diluted mastermix. A correction factor of 1.41 was applied to hydrolyzed sample and control results to reflect 100% naloxone hydrolysis in place of a protracted incubation time. The reporting cutoff for this assay was set above the method limits of detection and quantitation (LOD/LOQ) to accommodate the use of the correction factor. Preliminary sample data showed nornaloxone was only present in trace amounts relative to naloxone and naloxol concentrations. As a result, nornaloxone was excluded from the final validated method. Both naloxone and naloxol were found to have LODs/LOQs of 5 ng/mL. Upper limits of linearity and carryover were 10,000 ng/mL for both analytes. Inter/intra-assay precision did not exceed 6.5% coefficient of variation and accuracy was within 12.5% of the target concentrations for the two compounds. Matrix effect was -1.9% for naloxone and -32.4% for naloxol. All 36 patient samples tested were positive for naloxone (concentration range 8.1 - 9623.5 ng/mL) with 19 of them being positive for naloxone and naloxol. When detected, naloxol was typically at significantly lower concentrations than naloxone (concentration range 0.0 - 186.6 ng/mL). This is consistent with previous research indicating that naloxone would be the dominant urinary constituent post-hydrolysis.

Conclusion: The IMCSzyme™ recombinant beta-glucuronidase was a satisfactory choice for this assay. Naloxone appears to be most prevalent in urine relative to the other metabolites monitored. Application to patient sample analysis was 100% successful in detecting positivity of naloxone in all of the Suboxone® patient samples. The dilute and shoot method is robust on the Waters Acquity UPLC TQD platform, even with enzyme present, and was instituted with relative ease.

Keywords: Naloxone, Hydrolysis, Urine

P29

Short Term Stability Study of Synthetic Cannabinoids

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Background/Introduction: Synthetic cannabinoids designed to replicate the intoxicating effects of marijuana have been evolving over the past decade in an attempt to avoid detection. As compounds of abuse are identified, legislation has passed to ban specific molecules. In response, manufacturers have broadly diversified the functional groups and substituents to retain activity but continue to evade detection. While several research efforts have focused on identifying which cannabinoids are present in herbal formulations, there has been a lack of testing for compound stability and the potential for degradation between manufacturing and consumption. Given the number of emergency room reports related to synthetic cannabinoid-containing product exposure, understanding the chemical constituents and their potential for degradation during their manufacture, distribution and use is increasingly needed. Herein, we use Ultra Performance Liquid Chromatography – Ultraviolet – Mass Spectrometry (UPLC-UV-MS) to characterize parent compounds and their degradation products as they occur under a variety of environmental conditions.

Objective: The objective of this study is to characterize the chemical stability and degradation of synthetic cannabinoids during exposure to aqueous, basic, acidic, and oxidizing conditions, as well as at elevated temperatures.

Method: Compounds were dissolved in either acetonitrile, methanol, or DMSO. The solution was then diluted 50:50 with water, 3% hydrogen peroxide, 0.2N hydrochloric acid, or 0.2N sodium hydroxide and stored in a 25 °C 60% relative humidity chamber. Samples stored in 50:50 water/organic composition were also stored in an oven at a temperature of approximately 50 °C to assess thermal degradation. Immediately after preparation, an aliquot was taken from each forced degradation condition, diluted in organic solvent and stored at -80 °C until removal for analysis. Every 24 hours another aliquot was taken and stored in the same fashion. After five days of sampling, the solutions were analyzed by UPLC-UV-MS using a Waters Acquity UPLC coupled to a Synapt G2 Q-TOF system using water and methanol with formic acid as mobile phase and an Acquity UPLC BEH C18 1.7µm 2.1 x 50 mm column. A reference standard solution was also stored at - 20°C, from which a standard was made up on the day of analysis. Peak areas were compared over the five days by monitoring the UV trace, and both UV and MS data were used to determine loss of parent compound and the identity of chemical degradation products.

Result: The most extensive and rapid loss in parent signal was from transesterification of ester containing compounds (e.g. PB-22) when they were exposed to methanol in basic conditions. Additional significant changes in signal were seen for compounds containing a tetramethylcylclopropyl group (e.g. UR-144), in which the ring breaks open, resulting in a degradation product with the same mass, but a shift in retention time. Of the thirty seven unique compound and organic solvent combinations tested, sixteen (43%) did not exhibit any form of degradation, seventeen (46%) showed some form of loss of parent signal in one or more condition, and four (11%); JWH-018 Adamantyl Analog, AM2201 Benzimidazole Analog (FUBIMINA), 5F-THJ, and FDU-PB-22; showed degradation in every condition tested.

Conclusion: As structures classified as synthetic cannabinoids continue to diversify, it has become increasingly apparent that structural changes can dramatically alter their chemical stability and the chemical exposures occurring during their use. The chemical stability of these drugs can have important implications for handling, storage, use, and analysis; since the chemicals are often dissolved in an organic solvent before application to the plant material, and heated or combusted during use.

Keywords: Synthetic Cannabinoids, Designer Drugs, UPLC-MS

P30 WITHDRAWN

P31
Determination of an Alternate Source of Methcathinone in a Patient Sample

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Introduction: Clinical Reference Laboratory has been performing analysis of synthetic cathinones (aka "Bath Salts") since 2011. In early 2015, a positive result was challenged by the donor who was taking Allegra-D® extended release (containing fexofenadine and pseudoephedrine) at the time the sample was collected. Due to the challenge, further testing was performed in order to determine whether the pharmaceutical product in use was the actual source of methcathinone.

Objective: To determine the presence of methcathinone in the pharmaceutical product Allegra-D® and whether pseudoephedrine positive samples were also positive for methcathinone.

Method: Methcathinone confirmation in urine was performed using a Sciex API6500 QTrap LC/MS/MS for detection. The method employs a Waters Cortecs C18+ column (2.7 μm, 2.1 x 100 mm) and a mobile phase gradient with 10 mM ammonium formate, 0.1% formic acid in water, and methanol. The analysis is performed using dilute-and-shoot methodology. Analysis of a sample of Allegra-D® obtained by the lab was performed by extracting the compounds from a crushed tablet in a 3:1 0.1 N HCl:methanol solution, sonicating for fifteen minutes, and letting stand for 2 hours. Determination of methcathinone in the tablet extract was completed with the confirmation method used for analysis of the donor urine sample. Each tablet contains 60 mg of fexofenadine and 120 mg of pseudoephedrine in a 12 hour extended release formulation. Further analysis was performed using the API6500 QTrap in order to provide additional confirmation of the identity of methcathinone in the donor sample. A full scan product ion spectrum was generated using methcathinone standard and compared to the full scan product ion spectrum of the peak in the donor sample. Ephedrine and pseudoephedrine analysis was performed using a Sciex API2000 LC/MS/MS to confirm the presence of pseudoephedrine in the donor sample.

Result: Methcathinone was found in the donor urine sample at a concentration of 120 ng/mL. When this was reported, the donor challenged the result and indicated the use of Allegra-D® extended release at the time of collection. The product ion trap analysis of the peak in the chromatography matched the library spectrum for methcathinone with 80% purity which exceeds the laboratory's purity criteria of greater than or equal to 70%. The donor sample tested positive for pseudoephedrine at 179,000 ng/mL. From the extract of the Allegra-D® tablet, methcathinone was found at approximately 1.8 μg per tablet indicating that the tablet was a source of methcathinone. For additional evidence that methcathinone is a potential impurity in products containing pseudoephedrine, a standard solution obtained from Cerilliant containing 1 mg/mL of pseudoephedrine was also tested for methcathinone. The standard was found to contain 7 ng/mL of methcathinone which would be equivalent to 0.84 μg of methcathinone per 120 mg of pseudoephedrine. Three additional donor samples that were positive for pseudoephedrine were tested for methcathinone. The pseudoephedrine values ranged from 38,700 ng/mL up to 570,000 ng/mL for these samples. Methcathinone was confirmed in all samples with values from 23 ng/mL to 74 ng/mL. The ratios of methcathinone to pseudoephedrine in donor samples were at least ten times the ratio found in the tablet (0.0015%) or Cerilliant standard (0.0007%). An explanation for the changes in the ratios could be differences in absorption and elimination of the two compounds over the course of treatment which may require multiple doses over several days. Based on these results, the MRO reported the sample as negative for methcathinone.

Conclusion: Allegra-D® contains methcathinone at less than 0.1% of the active ingredient. Other products containing pseudoephedrine should be evaluated for the presence of methcathinone. Donor urine samples that are found to contain methcathinone must also be tested for pseudoephedrine in order to prevent possible false positive results.

Keywords: Methcathinone, Bath Salts, Synthetic Cathinones, LC/MS/MS, Quadrupole-Ion Trap

P32

Pathological Findings in Amphetamine-Type Stimulants Related Death

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Introduction: The number of users and ATS-related deaths are increasing by 1.7% from 2011 to 2012.(1, 2) ATS-related death are mostly cardiovascular-related, however it is not always the case, with abnormalities also found in other organs, such as liver or brain.(3)

Objective: The objective of this study is to understand what pathological conditions are found in ATS related death and what proportion of the abnormalities are as consequences of ATS abuse.

Method: Data was collected from the post-mortem case database at Forensic Medicine and Science, University of Glasgow. The cases selected were those positive for ATS between April 2007 and April 2015, regardless of cause of death. Five ATS were included in the criteria: Amphetamine, Methamphetamine, Methylenedioxymethamphetamine (MDMA), paramethoxyamphetamine (PMA) and paramethoxymethamphetamine (PMMA). Of all cases with positive ATS, only post-mortem cases with blood or urine concentration greater than 0.10mg/L were included. Detailed information of pathological findings and cause of death were identified, then.

Result: A total of 185 cases fulfilled the criteria. These cases consisted of 135 males (73.0%) and 50 females (27.0%). Age ranged from 15-66 years with a median of 38 years. Four ATS were found, namely: amphetamine, MDMA, PMA and PMMA with 155 cases (84%) were positive for amphetamine, 57 cases (31%) were positive for MDMA, 10 cases (5%) were positive for PMA and 8 cases (4%) were positive for PMMA. There were no cases positive for methamphetamine.

The number of cases with ATS listed as the cause of death was 84 cases (45%), though in just half of these (49 cases) has ATS as the sole cause of death. The causes of death of the other 35 cases were ATS combined with other drugs, such as cocaine, heroin or methadone. There were 26 cases where natural disease was also implicated in the cause of death.

In 33 cases (17.8% out of 185 cases) the cause of death was other drugs and in the rest of the cases (68 cases/36.8%) was not drug related, e.g. suicide.

Abnormalities were found in 6 organs: brain, lung, heart, aorta, liver and kidney. More than 25 abnormalities were observed in all the cases. The main findings were fatty change in the liver (31.7%), intra-alveolar haemorrhage (29%), atheroma in the aorta (25.3%), severe atheroma in the coronary artery (19.3%), contraction bands of the myocardium (14.0%) and brain haemorrhage (7.1%).

Conclusion: Whilst there are a number of deaths in which ATS are positive and may have caused death, in most individuals, the pathologies found are probably not related to ATS use. In a relatively small number of cases, pathologies, such as haemorrhagic stroke, associated with ATS use were identified and may have contributed to death.

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Keywords: ATS, Abnormalities, Post-Mortem

P33

Postmortem Tissue Distribution of AB-CHMINACA Following Lethal Intoxication Compared with AB-CHMINACA Concentrations in Impaired Drivers

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Introduction: N-[(1S)-1-(aminocarbonyl)-2-methylpropyl]-1-(cyclohexylmethyl)-1H-indazole-3-carboxamide, also known as AB-CHMINACA, is a heterocyclic indazole-based synthetic cannabinoid (SC). AB-CHMINACA and other SCs have psychoactive and pharmacological effects similar to delta-9-tetrahydrocannabinol and continue to have popularity as recreational drugs. Unlike Δ^9-THC, these drugs have severe toxicities and reported deaths. Recent studies indicate these cannabimimetic agents exert their pharmacologic effects as potent agonists at the cannabinoid 1 (CB_1) and cannabinoid 2 (CB_2) receptors. As of January 2015, AB-CHMINACA is a DEA *Schedule* I drug.

Objective: This report describes two post-mortem AB-CHMINACA cases, one single and one multiple drug intoxication, and three driving under the influence of drugs (DUID) cases involving AB-CHMINACA impairment.

Method: The first decedent was a 23 year old, white male recently released from a rehabilitation facility for marijuana abuse; he was last known alive about 5 hours before being found dead in the driveway at home.

The second decedent was a 33 year old white female with a history of past heroin abuse and current methadone treatment. She was found at home in a state of moderate decomposition. She was last known alive two days earlier when leaving a party.

The impaired drivers were males 21-34 years of age. The first two individuals were stopped by police for erratic driving and the third individual drove into a home. AB-CHMINACA and SC concentrations were measured in the various tissues: blood, urine, vitreous, gastric, liver, kidney, brain, bile and hair. Comprehensive toxicology and drug chemistry analyses were performed on multiple specimens and drug exhibits using gas chromatography/mass spectrometry.

The detection of SCs and AB-CHMINACA in tissues and fluids was accomplished using ultra performance liquid chromatography/tandem mass spectrometry after liquid-liquid extraction and in hair using high performance liquid chromatography/tandem mass spectrometry after solid-phase extraction.

Result: The postmortem values of AB-CHMINACA are shown in the table below.

Specimen (ng/mL)	Femoral Blood	Heart Blood	Urine	Gastric	Vitreous	Bile	Liver (ng/g)	Kidney (ng/g)	Brain (ng/g)	Hair
Case #1	7.0	16.9	*Pos	59.2 ng / 40mLs	Negative	46.7	404	NTDN	NTDN	NTDN
Case #2	7.1	7.8	NTDN	NTDN	NTDN	NTDN	115	26.5	21.3	Pos

NTDN=No testing performed. *AB-CHMINACA mono-hydroxyl metabolite

Case #1: No other drugs, including synthetic stimulants, were found to be present, other than caffeine and cotinine in the femoral blood.

Case #2: Consecutive 30-day hair segments from the root end were found to contain AB-CHMINACA at 29.9, 25.9 and 37.2 pg/mg. In the femoral blood Methadone and EDDP were present at 167 and 29.6 ng/mL, diphenhydramine < 0.05 mg/L, β-phenethylamine and cotinine reported as present.

DUID cases: For the three driving cases from Cuyahoga and Lake Counties, AB-CHMINACA was present in the peripheral blood at 10.8 ng/mL along with 0.20 ng/mL AB-PINACA, in the first driver, and 1.4 ng/mL of AB-CHMINACA in the second driver.
AB-CHMINACA was reported as present in the third driver. No other drugs or other SCs, than noted, were detected in the three DUID cases. Drug Chemistry exhibits were found to contain AB-CHMINACA in both postmortem cases. AB-FUBINACA was present in exhibits for the first driver and AB-CHMINACA for the second driver.

Conclusion: The cause of death in case #1 was ruled a single drug "acute intoxication by AB-CHMINACA" resulting in cardiac arrhythmia. AB-CHMINACA was distributed among multiple tissues with values ranging from 7.0 ng/mL femoral to 404 ng/g liver. Tissue and fluids associated with detoxification had higher concentrations of AB-CHMINACA. The cause of death in case #2 was ruled an "acute intoxication by the combined effects of AB-CHMINACA, methadone and diphenhydramine" resulting in cardiac arrhythmia. The manner of death for both cases was "accidental". All the DUID drivers were deemed "impaired" based on a standardized field sobriety test. The lethal and the impairment/DUID range of AB-CHMINICA appears to overlap in these cases.

Keywords: AB-CHMINACA, Synthetic Cannabinoids, Postmortem, Impairment

P34

ELISA with Reflex to LC-MS/MS for Detection and Quantitation of Buprenorphine in Meconium

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Introduction: Buprenorphine was approved for treatment of opioid addiction in 2002. Studies have shown it to be a safe and effective treatment for pregnant women (1). Babies born to mothers prescribed buprenorphine instead of methadone required less treatment for Neonatal Abstinence Syndrome (NAS). Kacinko et al demonstrated a correlation between meconium buprenorphine concentrations and neonatal outcomes (3). As such, buprenorphine concentrations in meconium could be used to guide newborn treatment and verify the mother's compliance with prescribed therapy. An enzyme-linked immunosorbent assay (ELISA) screen and confirmation test by LC-MS/MS were developed and validated for buprenorphine and norbuprenorphine in meconium.

Objective: To develop and validate a method to screen and confirm meconium specimens for buprenorphine and norbuprenorphine.

Method: An ELISA kit (part #236-0096, Immunalysis, Pomona, CA) was validated for meconium. An LC-MS/MS confirmation test for total buprenorphine and norbuprenorphine in meconium was developed and validated. Pre-analytical enzymatic hydrolysis and solid phase extraction were performed in the confirmation test. Both method validations met College of American Pathology (CAP), Clinical Laboratory Improvement Amendments (CLIA) and New York Department of Health (NYDOH) standards. The validated methods were used to evaluate 1,573 consecutive residual meconium specimens submitted to ARUP Laboratories for routine meconium drug testing. Results were de-identified following a University of Utah Institutional Review Board (IRB) protocol.

Result: The cutoff for the ELISA assay was 40 ng/g. 56% of samples spiked at the cutoff (n=2 over seven days, n=14 total) screened positive. Samples spiked at 50% (n=4 over seven days, n=28 total) and 70% (n=14) of the cutoff screened negative; samples spiked at 130% (n=14) and 150% (n=28) of the cutoff screened positive. Average imprecision for the ELISA assay was within ±20%. The cutoff for the LC-MS/MS assay was 20 ng/g. The upper limit of quantitation (ULOQ) was 1,000 ng/g. Accuracy of calibrators, QC, and spiked samples were within ±10% of expected values for buprenorphine and norbuprenorphine. Average imprecision (%CV) was within ±15%. Recovery ranged from 79-102% for both compounds. Matrix effect was 25%. 70 of 1,573 meconium specimens screened positive (4.5%) and were reflexed to the confirmation test. 66 screen positive samples (94%) confirmed for buprenorphine and/or norbuprenorphine. Four specimens failed to confirm, but norbuprenorphine was observed below the confirmation cutoff for two of these specimens.

Table 1. Confirmation Results (ng/g) for confirmed positive meconium specimens

	buprenorphine	norbuprenorphine
minimum	22	24
maximum	>1000	>1000
median	74	213
average	151	275
n	42	66

Conclusion: A cutoff of 20 ng/g for total buprenorphine and norbuprenorphine is appropriate for confirming ELISA-based screen results at a cutoff of 40 ng/g in meconium.

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Keywords: Buprenorphine in Meconium, ELISA, LC-MS/MS

P35

The Analysis of Synthetic Cannabinoids and their Metabolites in Human Urine by LC-MS/MS

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Background/Introduction: Synthetic cannabinoids are man-made chemicals, functionally similar to Δ9-tetrahydrocannabinol (THC), the psychoactive component in cannabis. They are applied onto plant materials and marketed as a "legal" high. The effects of synthetic cannabinoids are wildly unpredictable due to a consistently changing chemical makeup and have become a growing public health risk. The determination of cannabinoids and their metabolites, from a natural or synthetic source, has become routine in many forensic toxicology laboratories. The optimization of analysis time, resolution between metabolites, and method robustness 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 produce simple dilute and shoot methods with analysis times of less than 7 minutes for cannabinoids and their metabolites in urine.

Objective: Provide a fast and easy method solution for 17 synthetic cannabinoids and 12 metabolites resulting in complete resolution of isobars and separation from matrix interferences in diluted urine.

Method: The chromatographic method development investigation was performed on a Waters Acquity I-class equipped with a Xevo TQ-S using electrospray ionization in positive ion mode. An aliquot of 25 µL of the 10 ng/mL urine standard, 25 µL of the 25 ng/mL internal standard solution, and 350 µL of 0.1% formic acid in 50:50 water:acetonitrile were added to a 0.2 µm PVDF Thomson SINGLE StEP® Filter Vial 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 using water and acetonitrile mobile phases modified with 0.1% formic acid under gradient conditions on a Restek Raptor™ Biphenyl 2.7µm, 50 x 3.0mm column.

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. These isomers form because each parent compound has many sites available for hydroxylation. 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.

All the isomeric analytes included in this method were resolved on the Raptor™ Biphenyl column. By chromatographically separating these isomers, the most abundant metabolites from a given parent compound can be identified in authentic samples and methodology can be further optimized specifically for metabolites of clinical significance.

Conclusion/Discussion: The analysis of synthetic cannabinoids and their metabolites can be a difficult and challenging task. Many laboratories face the difficult task of developing and validating methods while keeping up with the ever-growing list of synthetic cannabinoids illicit drug makers produce. The Raptor™ Biphenyl provides solutions to many issues surrounding this analysis. It has the ability to provide highly retentive, selective, and rugged reversed-phase separations, allowing for the simultaneous analysis of 17 synthetic cannabinoids and 12 metabolites. 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: Cannabinoids, Metabolites, LC-MS/MS, Chromatographic Separation, Raptor™ Biphenyl

P36

Xylazine as a Drug of Abuse: Toxic Effects to Endothelial Cells in Speedball Combination

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Background: The use of xylazine in Puerto Rico and worldwide, as a drug of abuse and its combination with cocaine and/or heroin, has increased in recent years. FDA approves Xylazine for animal use only. Clinical findings reported that xylazine users presented with limb skin lesions, ulcerations and greater physiological deterioration than heroin users only.

Objective: The aim of this study was to assess the cytotoxicity of xylazine on endothelial cells, as this is the first tissue affected upon administration.

Method: Human umbilical vein endothelial cells in culture were treated with xylazine, cocaine, heroin and their combinations from 10 nM to 400 μM at 24, 48 and 72 hours exposure periods. IC_{50} was determined by applying Presto Blue reagent, a fluorometric assay, for viability determination. The Annexin V assay was also implemented as well as activation of caspases 8 and 9 in order to determine apoptosis as the cell death mechanism.

Result: Results indicated IC_{50} values at 24 hours as follow: xylazine 62 μM, cocaine 202 μM, heroin 278 μM and the combination of the three drugs 55 μM. Similar IC_{50}s was observed at 48 and 72 hours. Cases related to xylazine toxicity reported human plasma concentrations in a range among 1.0 μM and 20 μM, these cases weren't considered as drug users. The xylazine concentration on plasma users could be higher than these reported cases, considering that an epidemiologic study reported an increase in the frequency of injection since beginning to use xylazine (4-7/daily), which means that xylazine concentrations used in this study are comparable, to the doses used by addicts. These concentrations account only for the parent drug, metabolites concentrations were not determined. The Annexin V positive results as well as the positive activation of caspases 8 and 9 strongly suggest apoptosis as the cell death mechanism.

Conclusion: The study demonstrated that xylazine inhibits endothelial cell proliferation at lower concentrations than cocaine and heroin. These findings support that xylazine use increases the toxicity of cocaine and heroin when used in combination and induces cell death by apoptosis.

Keywords: Xylazine, Cocaine, Heroin, Apoptosis, Drug Abuse

P37
Quantitation of Ethylene Glycol in Postmortem Specimens by GC-TQ-MS/MS

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Background/Introduction: Ethylene glycol (EG) is a colorless, odorless, and sweet-tasting industrial product typically found in antifreeze that, if ingested, may cause severe toxicity and/or death. The extensive metabolism of EG in the body can lead to metabolic acidosis, cardiopulmonary failure, and acute renal failure, resulting in hundreds of deaths per year nationwide. Previously published methods for identification and/or quantitation of EG have used flame ionization detection (FID) or single-quadrupole mass spectrometric detection using single ion monitoring (SIM). An updated approach utilizing triple-quadrupole mass spectrometric detection (TQ-MS/MS) to identify and quantify EG in postmortem specimens was desired.

Objective: The objective of this study was to develop and validate a method for the simultaneous identification and quantitation of EG in postmortem specimens by GC-TQ-MS/MS following SWGTOX guidelines.

Method: EG and a deuterated internal standard (EG-d_4) were extracted from diluted calibrators, controls, and biological specimens with cold acetonitrile followed by the addition of an 80:20 mixture of 2,2-dimethoxypropane:DMF (400 µL). Samples were dried to ~200 µL under a nitrogen stream on a heating block at 80°C. A derivatizing reagent (100 µL of MTBSTFA/1% t-BDMCS) was added at room temperature to derivatize the hydroxyl functional groups present on EG and EG-d_4 for improved chromatography. Ethyl acetate (1.5 mL) was added to the derivatized samples and a 1-µL aliquot was injected onto the GC column (30 m x 0.25 mm i.d. x 0.25 µm Agilent DB-5MS). A Bruker SCION TQ-MS/MS with time-scheduled multiple reaction monitoring (MRM) was used for mass spectral identification and quantitation.

This validation followed SWGTOX guidelines to evaluate a linear calibration model, limit of quantitation, limit of detection, precision, bias, carryover, dilution integrity, selectivity, and sample stability. Crosstalk effect was also evaluated. This evaluation was necessary due to the MRM daughter spectra of EG and EG-d_4 being similar, despite using different parent ions for the time-scheduled MRM transitions.

Result: Linearity was verified from 25 to 2000 mg/L using a $1/x^2$ weighing factor with an operating limit of detection of 10 mg/L. Inter-day and intra-day precision at 50 mg/L and 1000 mg/L were both ≤1.0% with respective average biases of -2.0% and 2.6%. No carryover was observed in a blank sample following the injection of the 2000 mg/L calibrator. Precision and bias remained within 5% when evaluating diluted specimens at a 1:1 dilution with deionized water. No interferences with EG were observed when analyzed in conjunction with 80+ compounds, including ethanol, propylene glycol and glycolic acid. Samples remained stable over a 12-hour period. No crosstalk was observed between the m/z 237.0>147.0 (EG-d_4) and m/z 233.0>147.0, m/z 233.0>149.0, and m/z 233.0>73.0 (EG) MRM transitions.

Conclusion/Discussion: A novel analytical method was developed and validated for the simultaneous identification and quantitation of EG by GC-TQ-MS/MS using EG-d_4 as an internal standard. This method has demonstrated to be highly effective for the identification and quantification of EG in various postmortem and proficiency specimens. The technological advances in triple-quadrupole design over the last decade has allowed for faster scanning speeds and absence of crosstalk between MRM transitions, permitting further development of newer analytical methods. This method has been used by the Miami Dade Medical Examiner Department to quantify EG in over a dozen postmortem and proficiency cases that had only been previously qualitatively identified since 2012.

Keywords: Ethylene Glycol, GC-MS/MS, Method Validation

P38

Investigation into the Applicability and Reproducibility of MS/MS Spectral Data for the Identification of Designer Drug Regioisomers by LC-QTOF-MS

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Background: Due to the nature of designer drugs, there are many regioisomers present among the different designer drug classes. Regioisomers can complicate the identification of a drug in a toxicological analysis as they have the same molecular formula and produce similar if not identical product ions. Although chromatographic separation of regioisomers is preferred, it is not always practical, especially when trying to create a comprehensive screening method for hundreds of designer drug entities. While creating a MS/MS library for a comprehensive screening method, differences between the relative abundance of product ions were observed among sets of some designer regioisomers. If these differences are found to be reproducible then this could be a quick way to differentiate these regioisomers.

Objective: The objective of this study was to evaluate the applicability and reproducibility of using high resolution MS/MS spectral data generated by collision induced dissociation (CID) to identify specific regioisomers in a toxicological analysis. Fragmentation patterns and relative abundances of the product ions were evaluated to determine if there were any differences between regioisomers that could be used to identify a specific regioisomer from another.

Method: Fifteen sets of regioisomers (total of 38 drugs) from different designer drug families were used in this study. Each set consisted of two or three regioisomers. The analysis employed an Agilent 1290 Infinity High Performance Liquid Chromatography (HPLC) system coupled to an Agilent 6530 quadrupole-time of flight mass spectrometer (QTOF-MS) with an electrospray ionization (ESI) source. Standardized source conditions and three fixed collision cell energies (10, 20, and 40 eV) were used to collect MS/MS spectral data. Using flow injection analysis, neat drug standards were injected five times per day on three different days to determine the intraday/interday reproducibility of the fragmentation patterns and the relative abundances of individual products ions. This was also done to determine if there were any unique product ions or relative abundances of product ions that could differentiate regioisomers from each other. After product ions of interest were identified, multiple concentrations and mobile phase conditions were used to determine if the previously observed trends could be reproduced in different experimental conditions. In addition spiked blood and urine samples were extracted by solid phase extraction (SPE) and then analyzed by a targeted LC-QTOF-MS method to determine if the trends of the neat drug standards were consistent with extracted drugs. Analysis of variance was performed to determine if there were significant differences between the relative abundances of selected product ions.

Result: In each set of regioisomers one to six product ions were identified as ions of interest that could potentially be used to distinguish regioisomers. After evaluating the reproducibility under various conditions, nine of the fifteen sets of regioisomers contained at least two product ions that could be used to identify a specific regioisomer. Four sets of regioisomers did not have a product ion that was unique to a specific regioisomer. These observed trends were consistent in the extracted spiked blood and urine samples.

Conclusion/Discussion: Identification of designer drugs in a comprehensive analysis is increasingly important in forensic toxicology. When chromatographic separation is not practical, MS/MS spectral data could be used to differentiate regioisomers from each other based on their fragmentation pattern and the relative abundance of their product ions. While this may not be possible for every regioisomer, it could be used to quickly identify individual regioisomers were a significant difference exists in the fragmentation pattern and relative abundance of related regioisomers.

Keywords: Collision-Induced Dissociation, LC-QTOF-MS, Regioisomers

P39

Validation of Customized Biochip Array to Screen for Thirteen Drugs of Abuse in Blood from Randox Toxicology

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Background/Introduction: In 2013, the Mesa Police Department Forensic Services Toxicology Unit purchased a Randox Evidence Investigator to replace its current screening instrument for the preliminary screening of blood samples for drugs. A custom biochip array (named Drugs of Abuse XXI) was designed by Mesa Forensics, which included thirteen assays.

Objective: To validate the Mesa Forensics customized biochip array Drugs of Abuse XXI for the use of drug screening blood samples in driving under the influence of drug casework. The customized array contained the following assays: Ketamine, Fentanyl, Oxycodone, Hydrocodone, Zolpidem, Meprobamate, Meperidine, Zaleplon, Zopiclone, 7-aminoflunitrazepam, Trazodone, Tramadol, and Methylphenidate.

Method: The Randox Drugs of Abuse XXI custom array in conjunction with the Randox Evidence Investigator was evaluated for specificity, selectivity, reproducibility, robustness, accuracy, precision, limit of detection, linearity, quality control and sources of error. The SWGTOX Standard Practices for Method Validation in Forensic Toxicology were used to guide the validation. Samples were analyzed per the manufacturer's suggested method, using a sample size of 50uL. The sample aliquot then underwent a ¼ dilution with assay diluent, followed by centrifugation of 4000 RPM for 20 minutes. Precision at the decision point was evaluated by the analysis of fortified whole blood at the cut-off concentration as well as 50% above and below that level. These were tested over at least three runs, by at least two different analysts, with at least three replicates on each run. The results were compiled and the coefficient of variation was calculated for each analyte. Accuracy was assessed by determining the average percent recovery of the samples at the cut-off . The limit of detection was determined by replicate analyses (n=24) of negative whole blood over three separate runs.The average result was multiplied by 3.3 for each assay. Linearity was evaluated by graphing the relationship between relative light units (the measurement of the chemiluminescent reaction) and concentration. A correlation study was also performed using previously confirmed casework samples for the analytes of interest.

Result: The validation showed that the precision at the decision point for all assays had a coefficient of variation ranging between 9% and 26%. The limit of detection for all assays did not overlap with the cut-off levels. The percent recovery for the assays was found to vary widely, ranging between 63%-136%. Linearity showed a clear correlation between relative light units produced and concentration of analyte present in the sample. Correlation between the Randox results and the previously confirmed casework samples was consistent for seven of nine samples. Two samples screened positive for analytes that were previously not found, possibly due to screening/confirmation capabilities at the time that sample was originally tested.

Conclusion/Discussion: The Randox Drugs of Abuse XXI array in conjunction with the Randox Evidence Investigator was evaluated on the parameters of specificity, selectivity, reproducibility, robustness, accuracy, precision, limit of detection, linearity, quality control and sources of error. A correlation study was also carried with previously confirmed case samples. The Randox Drugs of Abuse XXI array allows for drug screening of a wide range of drugs with minimal sample volume. This aspect of this method was highly desireable as adequate sample volume is not always received for casework. The Randox Drugs of Abuse XXI array was approved for casework June 1, 2015.

Keywords: Randox, Immunoassay, Method Validation

P40

Clinical Indicators of THC Use as Shown Among Suspected Driving Under the Influence of Drugs (DUID) Arrestees from 2013-2015

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Introduction: A Drug Recognition Evaluation (DRE) is performed by a trained police officer on individuals suspected of driving under the influence of drugs (DUID). The National Highway Traffic Safety Administration (NHTSA) standardizes the testing used to evaluate these potentially impaired individuals. Potentially impaired individuals are evaluated using different tests to help ascertain which category of drugs may be causing impairment. Some of these tests include pupil size using different lighting conditions, lack of convergence (LOC), horizontal gaze nystagmus (HGN), pulse and blood pressure (BP), and the Romberg balance test. The specific clinical indicators observed in individuals suspected to be under the influence of THC are LOC, normal to slow reaction to light, elevated pulse, elevated BP, and body/eyelid tremors.

Materials and Method: Individuals arrested in Miami-Dade County suspected of DUID are required to submit a urine sample for drug analysis. These samples are delivered to the University of Miami toxicology lab for testing. The sample pool includes both male and female from various ethnic backgrounds and age groups. 11-nor-9-carboxy-Δ9-tetrahydracannabinol (THC-COOH) was determined by gas chromatography-mass spectrometry (GC/MS) in selected ion monitoring mode (SIM) using D6 11-nor-9-carboxy-Δ9-tetrahydracannabinol as the internal standard. A negative control, low control (15ng/mL) and high control (140ng/mL) are also analyzed alongside each batch. The limit of detection for this method is 5 ng/mL. Sample preparation is a liquid-liquid extraction (LLE) and subsequent derivitization with N-methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide (MTBSTFA). This method was validated in accordance with SWGTOX guidelines for interference, carryover and specificity.

Result: 312 positive THCA cases were evaluated from 2013-2015, of these only 23% had corresponding DREs and were included in this study. THC is the main psychoactive substance found in marijuana. However, the most frequently encountered metabolite in urine is THC-COOH which is an indicator of marijuana use. In total, from the 73 individual cases positive for THC-COOH, 68% demonstrated rebound dilation, 69% had increased blood pressure, 76% had a slow reaction to light stimulus, and 58% had visible eye tremors. Although only 58% had an increased pulse rate, the entire sample pool (100%) demonstrated lack of convergence.

Conclusion: As detection of THC-COOH in urine samples far exceeds the window of impairment, it is imperative to have indication of drug impairment from physiological data. The study results are consistent with the findings of NHTSA which states those potentially under the influence of THC can demonstrate an elevated blood pressure, reaction to light normal to slow, body/eyelid tremors, and have a lack of convergence present. The elevated pulse rate was seen in 58% of the population. This could possibly indicate that other substances may be affecting the pulse rate, as 52% of our cases tested contained other illicit and/or prescription drugs. All subjects demonstrated lack of convergence which strongly supports NHTSA fact sheets indicating recent use of THC. Interestingly, 68% of the cases also demonstrated rebound dilation which is not a sighted indication of THC use according to NHTSA, however was indicated as a marker in this population.

Keywords: THC, GC-MS, Convergence, DRE

P41

Evaluation of a New Homogeneous Enzyme Immunoassay for the Detection of Hydrocodone and Hydrocodone Metabolites in Human Urine

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Background/Introduction: Hydrocodone is a semi-synthetic opioid derived from codeine and thebaine. It is used to relieve moderate to severe pain and to treat a cough. We evaluated a new DRI® Hydrocodone assay intended for the qualitative and semi-quantitative detection of hydrocodone and its metabolites in human urine at a cutoff concentration of 300 ng/mL. The DRI® Hydrocodone assay reagents are liquid, ready-to-use, enzyme immunoassay.

Objective: The objective of this study was to evaluate a new homogeneous enzyme immunoassay for the detection of hydrocodone and its metabolites in human urine.

Method: This study was conducted using a Beckman Coulter model AU680 chemistry analyzer. Reagents, calibrators, and controls were supplied by Thermo Fisher Scientific (Fremont, CA). Two lots of DRI® Hydrocodone reagent were evaluated using qualitative and semi-quantitative instrument parameters supplied by the manufacturer. Evaluation included precision, calibration stability, recovery, and method comparison studies.

Result: The assay demonstrates excellent precision at and around the cutoff, with Coefficient of Variation (CV) less than 5% for all levels which included quality control targets of 225 ng/mL and 375 ng/mL. Calibration stability studies indicate that the instrument calibration remains stable for at least seven days (maximum evaluated) in both qualitative and semi-quantitative modes. Recovery studies conducted using quality control (QC) material exhibit complete analyte recovery. Method comparison studies using one hundred and fifty Liquid Chromatography Tandem Mass Spectroscopy (LC-MS/MS) verified positive and negative samples demonstrate 100% agreement among samples positive for hydrocodone and/or metabolites, and 90% agreement among samples negative for hydrocodone and other metabolites with an overall agreement of 95%. The seven discrepant samples were false positive results associated with samples containing high levels of Oxycodone and its metabolites. All results obtained in this study show no significant differences between two lots of reagent.

Conclusion/Discussion: The data obtained at DrugScan using two lots of DRI® Hydrocodone reagent on a Beckman Coulter AU680 analyzer indicates excellent specificity and sensitivity to hydrocodone and its metabolites. This assay also has good specificity to hydromorphone and hydromorphone glucuronide. This assay's high specificity bridges the gap between the generic not so specific opiate assays that are currently on the market. The method comparison study produced seven false-positive results in samples that were identified as negative for hydrocodone and metabolites by LC-MS/MS. These false positives occurred using both the qualitative and semi-quantitative instrument parameters. Of these seven samples, oxycodone, noroxycodone, and oxymorphone were present in all cases, indicating a possible cross-reactivity. The exact concentration of parent and/or metabolite necessary to trigger the false positive in this assay needs further evaluation. Known additional metabolites of oxycodone that were not measured using LC-MS/MS during this evaluation include α- and β-oxycodol, α- and β-noroxycodol, and α- and β-noroxymorphol. Overall, this assay provides clinical laboratories with an additional tool to monitor patient compliance and meet proposed new SAMHSA testing criteria and afford laboratories the ability to differentiate consumption of hydrocodone and metabolites from other commonly prescribed opioids.

NOTE: The assay is currently in development and is not approved by FDA

Keywords: Hydrocodone, Metabolites, Immunoassay, HEIA

P42

Evaluation of a Newly Formulated Enzyme Immunoassay for the Detection of Hydrocodone and Hydromorphone in Pain Management Compliance Testing

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Introduction: Hydrocodone is a widely used analgesic for treatment of acute and chronic pain management. Additionally, it has a high abuse potential, being subjected to widespread drug diversion by opiate abusers. Thus, hydrocodone and its main urinary metabolite, hydromorphone, are an integral part of pain management compliance testing (PMCT) and urine drug abuse (DOA) testing. Recently, a new Hydrocodone Enzyme Immunoassay [HEIA] (Lin-Zhi International, Inc., Sunnyvale, CA) has been released for the detection of hydrocodone and hydromorphone in PMCT and DOA testing. This assay is available in 100 ng/mL and 300 ng/mL hydrocodone cutoffs.

Objective: To evaluate the analytical efficiency of the new Lin-Zhi HEIA for detection of hydrocodone and hydromorphone in urine. The assay was used to screen over 1,000 urine specimens at both the 100 ng/mL and 300 ng/mL cutoff values. Possible interferences from cross-reacting compounds were also studied. These data will assist in determining the utility of a specific hydrocodone assay.

Method: HEIA testing was performed on an ARCHITECT Plus c4000 Clinical Chemistry Analyzer (Abbott Diagnostics, Abbott Park, IL). Its precision (intra-run and inter-run) were determined by analyzing control specimens containing ±25% for each of the 100 ng/mL and 300 ng/mL cutoff concentrations; intra-run (n=10) and inter-run (n=25), at five replicates per day for five days. Selectivity and sensitivity were evaluated at the 100 ng/mL and 300 ng/mL cutoffs by analyzing 1019 and 1025 urine specimens, respectively. The assays were calibrated each day with a 0 ng/mL drug-free calibrator and the cutoff calibrator, either 100 ng/mL or 300 ng/mL of hydrocodone. With each batch, quality control drug-free specimens and ±25% cutoff concentrations of hydrocodone were analyzed. All urines were post-diagnostic specimens from pain management patients previously screened by an opiate EIA (Microgenics Corporation, Fremont, CA) and analyzed by UPLC-MS/MS for opiates.

Result: The intra-run and inter-run precisions were CV ≤2.5%, for both cutoff assays. The 100 ng/mL HEIA yielded 34% (349/1019) positive results. Of these, 61% (214/349) were confirmed for hydrocodone and/or hydromorphone at ≥100 ng/mL; hydrocodone values ranged from 100 to 83,000 ng/mL and hydromorphone ranged from 100 to 112,000 ng/mL. The 300 ng/mL HEIA yielded 20% (207/1025) positive results. Of these, 77% (161/207) were confirmed for hydrocodone at ≥300 ng/mL and/or hydromorphone at ≥375 ng/mL; hydrocodone values ranged from 300 to 83,000 ng/mL and hydromorphone ranged from 380 to 112,000 ng/mL. False positive specimens contained a single opiate at very high concentrations or a mixture of those. These specimens accounted for 13% (135/1019) and 4.5% (46/1025) of the samples with 100 ng/mL and 300 ng/mL cutoffs, respectively. Oxycodone was present in 69% (124/181) at 100 to 55,000 ng/mL and morphine was present in 47% (88/181) at 400 to 240,000 ng/mL of the total false positive results from both assays. With the 100 ng/mL cutoff, oxycodone caused the majority of false positive results, while with 300 ng/mL morphine dominated. Other opiates were less frequently present. The selectivity and sensitivity for the 100 ng/mL cutoff was 83% and 96% and for the 300 ng/mL cutoff was 95% and 88%, respectively. The concordance of HEIA results and UPLC-MS/MS results for the 100 ng/mL and 300 ng/mL cutoff was 86% and 93%, respectively. The efficiency of the 100 ng/mL assay was 86%, while the 300 ng/mL was 90%.

Conclusion: The Lin-Zhi HEIA 100 ng/mL cutoff assay demonstrated to be sensitive for the detection of hydrocodone and hydromorphone in urine, while the 300 ng/mL was more selective, but not as sensitive. The HEIA has the potential to detect hydrocodone/hydromorphone in positive specimens screening negative by opiate EIA. Therefore, in zero tolerance testing in PMCT, a specific HEIA should be part of an initial immunoassay screen.

Keywords: Hydrocodone, Hydromorphone, Lin-Zhi Enzyme Immunoassay

P43

The Analysis of Common Antiepileptic Drugs in Human Urine by LC-MS/MS

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Background/Introduction: The use of liquid chromatography coupled with mass spectrometry (LC-MS/MS) in therapeutic drug monitoring and toxicology labs has increased significantly over the years. LC-MS provides sensitivity, speed, and the ability to simplify sample preparation. The Raptor™ Biphenyl column was developed to complement high-throughput LC-MS/MS analyses by combining the increased efficiency of superficially porous particles (SPP) with the resolution of Ultra Selective Liquid Chromatography™ (USLC™) technology. In this example, a simple dilute and shoot method was developed for 14 common antiepileptic drugs in urine using a Raptor™ Biphenyl column.

Objective: The intent of this study was to develop a simple dilute-and-shoot method for the analysis of 14 antiepileptic drugs in human urine with a rapid run time of 5.5 minutes.

Method: Human urine samples were diluted in 0.1% formic acid 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 ion mode with scheduled multiple reaction monitoring (MRM). The separation was performed using water and methanol mobile phases modified with 0.1% formic acid under gradient conditions on a Restek Raptor™ Biphenyl 2.7µm, 100 x 2.1mm column. Target analytes were: pregabalin, gabapentin, levetiracetam, zonisamide, felbamate, lacosamide, fosphenytoin, topiramate, ezogabine, phenytoin, tiagabine, eslicarbazepine, clobazam, perampanel.

Result: Linearity, precision, and accuracy experiments were performed during method development. Purchased human urine was fortified with 14 drug analytes and their deuterated internal standards. The calibration range for most analytes was from 10 to 1000 ng/mL; R values were all greater than 0.990. Accuracy and precision were determined by fortifying human urine at a concentration of 800 ng/mL prior to dilution. Mean values at this level ranged from 88% to 110% of nominal concentrations for all analytes. Coefficient of variation (CV) was calculated for the determination of precision and ranged from 6.2% to 10.5%.

Conclusion/Discussion: The Raptor™ Biphenyl column was excellent for the simultaneous analysis of 14 antiepilectic drugs in human urine. The accurate and reproducible analysis can be achieved in less than 5.5 minutes of chromatographic run time and is thus applicable for low-cost and high through-put analysis in therapeutic drug monitoring and toxicology labs.

Keywords: Antiepileptic Drugs, LC-MS/MS, Raptor™ Biphenyl

P44

The Role of Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) in Clinical and Forensic Toxicology Applications

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Background/Introduction: Analysis of trace element in biological matrix is essential. For example, lead is a neurotoxic metal that affects areas of the brain that regulate behaviour and nerve cell develpment.[1] Even 10 µg/dL lead level is associated with cognitive development and growth issues in young children. Cobalt (Co^{2+}), is regarded as a potential enhancing substrate in endurance sports, therefore analysis of Co^{2+} in urine could be an emerging tool for sports doping.[2] Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is a powerful tool for trace elemental analysis. It provides a fully quantitative technique for almost all elements in the periodic table, offers isotopic information and isotope ratio determinations. Therefore, developing sensitive ICP-MS methods for trace metal analysis in blood and urine is highly desired by the clinical and forensic toxicology community for reference use.

Objective: Develop sensitive ICP-MS methods for lead analysis in blood, and trace Co^{2+} analysis in urine.

Method: The Thermo iCAP Qc ICP-MS was used for acquisition of all data. Blood samples were diluted 100-fold in 0.5% HNO_3 prior to an additional 2x dilution from the online addition of the internal standard (1 ng/mL Bi in 0.5% HNO_3). To analyze blood samples, a SC-2DX autosampler equipped with a 7-port FAST valve was used. To analyze lead, the iCAP Q was operated in STD mode (QCell not used). Urine samples were diluted 20-fold in 2% HNO_3, and 50µl of internal standard solution (5 ng/mL ^{45}Sc, ^{73}Ge, 2.5 ng/mL ^{103}Rh and ^{193}Ir) was added to each sample. An SC-4Q autosampler was used to introduce urine samples for the total element quantification (TEQ). Single He KED mode was used for all measurements.

Result: The analysis of lead in whole blood is definitely challenging for ICP-MS due to its complex nature. Dilution of the sample was used to overcome these effects with well-preserved elevated detection sensitivity. For analysis lead in whole blood, the resulting method detection limit was calculated to be 0.02 µg/dL. The calibration range of this method is from 0.02-1 µg/dL. Considering the 100-fold dilution factor, this method allows to quantify lead in patient whole blood from 2-100 µg/dL, which satisfies the routine toxicology requirement range.[1] The accuracy of the method was also verified using Lyphochek™ Whole Blood Controls containing three different levels of lead. The results showed that for each level, the accuracy is within ±2%. The method robustness was achieved with implement of 209Bi internal standard. For 35 injections, the method recovery was well maintained within 90%-110%.[3] For trace Co^{2+} analysis in urine, 200 urine samples were analyzed in order to verify baseline levels of Co in urine. Furthermore, an elimination study was performed to verify that intake of Co^{2+} leads to an increase of the urinary Co^{2+} concentration. Results show that the Co^{2+} concentration in urine is increased to between 40 and 318 ng/mL within 6h after intake of Co^{2+}, whereas the normal concentration was observed to be between 0.1-2.2 ng/mL.[2] Intake of Vitamin B12 does not seem to affect the urinary Co^{2+} level.

Conclusion/Discussion: The iCAP Q ICP-MS is a sensitive instrument. In the current abstract, the method allows 100-fold dilution of the whole blood sample, but still preserves good calibration range from 2-100 µg/dL lead. This is a sensitive and robust ICP-MS method for determination of trace levels of lead in whole blood samples in the routine toxicology laboratory. The detection of cobalt (Co^{2+}) in urine has been shown to be a potential tool to investigate its abuse in sports doping. Further investigation is required to establish a robust testing method.

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Keywords: ICP-MS, Lead, Cobalt

P45

Comprehensive Toxicological Screening of Urine Samples by the Toxtyper™

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Background: Screening for drugs in urine samples with GC/EI-MS-FullScan and EI-MS library search can be regarded as standard practice in clinical toxicology. The Toxtyper™ (TT, Bruker) uses LC-MS^n ion trap technology with MS^n library search probably allowing a simplified sample preparation.

Objective: Compare the performance of the TT with our standard GC/MS procedure on the reported result level.

Method: EI-MS-FullScan (Shimadzu QP2010plus) with Maurer/Pfleger/Weber-library (4th Edition, Wiley-VCH) search was performed after enzymatic hydrolysis of 2 ml urine, alkaline liquid-liquid-extraction, acetylation and 16.5 min GC separation. For TT analysis 250 µL urine was protein-precipitated with 200 µL acetonitrile. The supernatant was evaporated to dryness and redissolved in 50 µL mobile phase A. All samples were analyzed with TT method 1 (TT-M1, run time 11 min) and TT method 2 (TT-M2, run time 19 min) according to Bruker. TT-M1 includes continuous positive/negative switching and generates MS2/MS3 spectra according to a scheduled precursor list. Substances were automatically identified with the TT-library (900 entries) by Rt and MS, MS2 and MS3 information. TT-M2 data (MS, MS2/MS3) were acquired in positive mode and searched against TT-library and the Maurer/Wissenbach/Weber-library (TT-MWW; Wiley-VCH, 2014). TT-MWW contains >4500 entries including 3000 metabolites and conjugates. Routine urine samples from 150 patients mainly in substitution therapy were analyzed.

Result: For the 150 urine samples 482 results were reported which could be attributed to 61 different drugs and/or their metabolite(s). GC/MS found 2 substances which were not detected by the TT. With TT-M2 9 substances not identified by GC/MS were found. In 352 of the 482 results (73%) TT and GC/MS gave according results (51 substances; TT-M1+TT-M2+GC/MS: 258, TT-M1+GC/MS: 19, TT-M2+GC/MS: 75). In 53 cases (11.0%) 14 different substances were identified by GC/MS which were not found with both TT approaches. However, 77 reports (16%) were based on TT analysis only (25 substances; TT-M1+TT-M2: 42, TT-M2: 34, TT-M1: 1). The 130 discrepant results were due to sensitivity differences of both methods for the 39 substances belonging to 15 different substance classes.

Conclusion: The presented TT screening analysis after simple sample preparation proved to be at least of equal value when compared to our standard GC/MS approach. Future improvements in TT sample preparation could lead to the use of TT-M2 only.

Keywords: Toxtyper™, GC/EI-MS-FullScan, Urine Screen

P46 ***"NIJ Funded"***

Expanded Compound Database and High Resolution MS/MS Spectral Library for the Detection of Designer Drugs by LC-QTOF-MS

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Background: High resolution, high mass accuracy (HRMS) techniques combined with chromatographic methods are powerful tools for the screening of novel psychoactive substances ("designer drugs"), especially in toxicology and forensic science settings. The LC-QTOF-MS approach offers MS/MS capabilities, which allows for greater sensitivity and higher confidence in both targeted and untargeted screening of compounds, such as designer drugs, in complex biological matrices. In addition, QTOF HRMS data can be used to identify novel, previously unknown substances and can be retroactively screened, thus eliminating the requirement for sample reanalysis.

Objective: The purpose of this project was to expand a previously developed compound database and high resolution MS/MS spectral library using LC-QTOF-MS instrumentation to include 750+ designer drugs and metabolites from multiple drug classes. A second aim was to screen authentic urines collected from subjects in drug treatment programs to determine prevalence of designer and other drug use in this cohort.

Method: An Agilent 1290 Infinity UHPLC system with a 6530 Accurate-Mass QTOF-MS with a Jet Stream Technology electrospray ion source (ESI) was used for this project. Drug standards were directly injected via flow injection analysis in ESI at a concentration of 1 μg/mL with a mobile phase that consisted of 50:50 5 mM ammonium formate with 0.1% formic acid in water and 0.1% formic acid in acetonitrile. MS/MS spectral library data were collected for the entire dataset at three different collision energies (10 eV, 20 eV, and 40 eV). Creation of the compound database involved identifying potential designer drugs from various published and unpublished sources. Each entry in the database included the following information: compound name, chemical formula, monoisotopic mass, chemical structure, and IUPAC name. ChemSpider and CAS numbers were included when available. The spectral library and database were created using MassHunter PCDL Manager software. Following a five-fold aqueous dilution, authentic urine specimens obtained from a private drug testing facility were screened by LC-QTOF-MS and the data processed using the MS/MS library and database for potential designer drugs present.

Result: This project is a continuation of ongoing development of a high resolution MS/MS spectral library and compound database from this research group. The new library added data for 499 additional designer drug standards and 76 deuterated internal standards, including spectral data for compounds from the FUBINACA, PINACA, and CHMINACA classes. The expanded database contains >150 stimulant compounds (phenethylamines, cathinones, and tryptamines) and >270 cannabinoids, and includes over 100 metabolites and related compounds. The spectral library and database can be used to aid in designer drug identification in various HRMS data collection modes, including full scan, targeted MS/MS, and auto MS/MS. To date, the library has been effective in screening a series of spiked urine specimens for a variety of novel psychoactive substances. In addition, the expanded library is currently being employed for screening of ~1000 authentic urine specimens obtained from subjects in pain clinics and enrolled in drug treatment programs.

Conclusion: An expanded high resolution MS/MS designer drug spectral library and compound database were created to improve the screening potential of the LC-QTOF-MS. The developed MS/MS library and compound database will be added to the existing library, bringing its size to 750+ designer drugs and related compounds. The combination of a high resolution MS/MS library and compound database can be useful for the identification of designer drugs in screening applications of forensic toxicology.

Keywords: Designer Drugs, High Resolution MS/MS Library, LC-QTOF-MS

P47 ***"NIJ Funded"***

Triggered MRM Database for the Comprehensive Detection of Novel Psychoactive Substances by LC-QqQ-MS

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Background: LC-QqQ-MS can be considered a useful targeted screening/confirmation/quantification tool in forensic toxicology due to its high sensitivity, MS/MS capabilities, and, depending upon the instrumentation, its ability to target up to 10 precursor-product transitions. This strategy enhances the potential (as compared to the more standard 2-4 transitions) to "fingerprint" target isomeric compounds typically present in complex chromatographic separations. LC-QqQ-MS is also less expensive and more readily available than high resolution instrumentation and currently many forensic laboratories have incorporated this technology in their workplaces. While various methods incorporating this approach have been validated for limited numbers of novel psychoactive substances (NPS), there are currently no comprehensive methods available for screening human specimens for the hundreds of NPS now in existence.

Objective: The goal of this project was to create a comprehensive tMRM database for 750+ novel psychoactive substances from multiple drug classes using LC-QqQ-MS instrumentation. A second aim was to screen authentic urines collected from subjects in drug treatment programs to determine prevalence of designer and other drug use in this cohort.

Method: An Agilent 1290 Infinity HPLC system with a 6460 QqQ-MS with an Agilent Jet Stream Technology electrospray ion source (ESI) was used for this research. Drug standards in methanol or other appropriate solvent at a concentration of 1 µg/mL were injected directed into the ESI using a mobile phase made up of 80:20 0.1% formic acid in methanol and 5 mM ammonium formate with 0.1% formic acid in water. Up to 10 transitions were monitored for each precursor ion, based on a minimum required peak intensity of 1000 counts for each product ion, using MassHunter MS Optimization Software. Both fragmentor voltage and collision energy were automatically optimized for each target drug by the optimizer software. To test the applicability of this screening method, authentic urine specimens were diluted five-fold in water and LC was performed on an Agilent Zorbax Rapid Resolution HD Eclipse Plus C18 column (3.0 x 100mm 1.8 micron particle size).

Result: This research is an expansion of a previously developed tMRM spectral dataset by this research group. The new tMRM database includes data from 575 standards, which includes >150 stimulants (phenethylamines, cathinones, and tryptamines) and >270 cannabinoids, including >100 metabolites, along with 76 deuterated compounds. Only precursor-product transitions with peak intensity above 1000 counts were included in this database. Any compound that resulted in less than 4 transitions above 1000 counts were excluded from the final database. Approximately 45% of all compounds analyzed showed 10 precursor-product ion transitions above 1000 counts. These findings will be able to assist in screening by LC-QqQ-MS and expand on the existing tMRM database. The expanded tMRM dataset is being employed in ongoing screening of ~1000 authentic urine specimens from subjects in drug treatment programs.

Conclusion: An expanded tMRM spectral dataset were created for novel psychoactive substances to enrich screening possibilities of the LC-QqQ-MS. The current expanded database includes 750+ novel psychoactive substances, metabolites, and associated compounds. The comprehensive tMRM spectral dataset can be beneficial to the identification of novel psychoactive substances in forensic toxicology screening.

Keywords: Novel Psychoactive Substances, MRM Database, LC-QqQ-MS

P48

Effective Extraction Strategies for Buprenorphine and Norbuprenorphine in Urine, Oral Fluid and Whole Blood Using Cation Exchange Solid Phase Extraction and Supported Liquid Extraction prior to HPLC-MS/MS Analysis

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Introduction: Buprenorphine and Norbuprenorphine are typically problematic for analysis due to analyte stability issues during sample preparation. A fast and reliable testing protocol is needed to address extracting the targets out of complex matrices typically encountered during toxicological testing. A fast, reliable and robust sample preparation method that could be implemented to extract these drugs from complex biological matrices with good analyte recovery and minimum matrix effects would be ideal for toxicology labs. Here, we demonstrate new rapid and reliable sample preparation methods that were used to extract the target analytes from small amounts of the biological matrix. These methods were fully automated on the Biotage Extrahara® Sample Preparation Workstation. Qualitative and quantitative data demonstrates the utility of these methods prior to LC-MS/MS analysis.

Method: Supported Liquid Extraction in a 96 fixed well plate format was used to extract Buprenorphine and Norbuprenorphine from whole blood spiked at concentrations from 1.0 -100 ng/mL. Sample pre-treatment consisted of a 1:3 dilution of blood (100µL) with 0.1% ammonium hydroxide (300µL). An optimal extraction solvent of ethyl acetate: acetonitrile: ammonium hydroxide was identified. Cation Exchange Solid Phase Extraction in a 96 well plate format was employed for the extraction of the same target analytes from urine and oral fluid (neat and buffered) spiked at a concentration range of 0.1-100ng/mL. Sample preparation consisted of a 1:9 dilution of the matrix with 0.1% Formic Acid. Both extraction processes were fully automated on the Biotage Extrahera® Sample Preparation workstation. Extracts were evaporated to dryness, re-constituted in mobile phase and injected onto an Agilent 1200 coupled to a Sciex 4000 Q-trap® triple quadrupole mass spectrometer for analysis.

Result: Averaged recoveries of greater than 90% was observed for the target analytes in urine and oral fluid. Averaged recoveries greater than 70% was observed for analytes in whole blood. All averaged recovery % RSDs were calculated at less than 10%. Measured matrix effects ranged from 8%-50% for the analytes in all three matrices. In-house calibrators were prepared in urine, blood and oral fluid matrices. Calibration curves were generated for the analytes across the dynamic range of 0.1ng/ml to 100ng/ml for samples in oral fluid and urine. Calibration curves were generated across the dynamic range of 1.0ng/ml to 100ng/ml for the analytes extracted from whole blood. All calibration curves were linear with $r^2 > 0.99$. The precision and accuracy of the in-house calibrators was determined to be within ±15%. The LOD for all three matrices was determined to be 0.1ng/ml and the LOQ for the analytes in urine and oral fluid was determined to be 0.2ng/ml. The LOQ for the analytes in whole blood was determined to be 0.5ng/ml.

Conclusion: We present a fast simplified approach for extraction of Buprenorphine and Norbuprenorphine with reproducible recoveries for low analyte detection levels (0.1ng/ml) using a minimal amount of sample (100 µL).

Keywords: Supported Liquid Extraction, Solid Phase Extraction, Drug Screening

P49

Development and Validation of a Method for the Determination of Nicotine in Postmortem Blood Using LC-MS/MS and a Case Report, Death of Nicotine Poisoning

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Backgound/Introduction: Nicotine is an alkaloid found in the Solanaceae plants and widely used as an insecticide in the past. Its median lethal dose (LD_{50}) is 50 mg/kg for rats, 3 mg/kg for mice and 30-60 mg (0.5-1.0 mg/kg) can be a lethal dose for adult humans. It acts as a stimulant in small amounts, but high doses can be harmful. A 56-year-old man was found dead in his office. Some search results for toxic substances (e.g. cyanides, etc) for suicide were found in his computer and nicotine was detected in postmortem specimens.

Objective: In this study, a simple, fast and reliable method for the determination of nicotine in postmortem blood was developed and validated.

Method: Acetonitrile was used for protein precipitation and supernatant was analyzed by high performance liquid chromatography coupled with electro spray ionization mass spectrometry. Analytes were separated by RESTEK Allure PFPP column (2.1 x 50 mm, 5 μm) using a flow rate of 0.5 mL/min to 1.0 mL/min at 35℃ with gradient elution. Mobile phase A was 2 mM ammonium formate in D.W and B was 2 mM ammonium formate in acetonitrile.

Result: Good linearity was obtained with correlation coefficients (R^2) of 0.996 for nicotine and the method was validated by evaluating the selectivity, precision, accuracy and recovery. The accuracy at three different concentrations was 95.2-120.8%, the precision was 0.81-2.93% and the recovery was 81.2-115.4%.

Conclusion/Discussion: In this case, nicotine was identified and quantitated on analysis of postmortem specimens; heart blood and peripheral blood. The concentrations of nicotine were 84 mg/L in heart blood and 58 mg/L in peripheral blood. This method can be successfully used to detect nicotine in biological samples.

Keywords: Nicotine, Alkaloids, Postmortem Concentrations

P50
Quantitative Determination of Oxytocin in Blood & Pharmaceuticals by LCMSMS

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Background: Oxytocin (OT) is a cyclic nonapeptide (CYIQNCPLG-NH_2) with a wide variety of therapeutic applications including stimulating labor, control of post-partum hemorrhage and induction of lactation. Recent studies proved that, when administered unnecessarily or in large doses it acts as antidiuretic, even at therapeutic doses it is responsible for major shifts in blood distribution. It also causes subcutaneous vessel vasodilatation and splanchnic bed plus coronary vessel vasoconstriction with a resultant drop in mean arterial pressure (MAP) while stimulating myocardial conductivity and heart rate [1]. On the other hand OT also demonstrated an inhibitory and amnestic action on learning and memory in different paradigms. Studies suggest the possible role of this neuropeptide in the regulation of drug abuse. Therefore, OT may act as a neuromodulator on dopaminergic neurotransmission in limbic-basal forebrain structures to regulate adaptive CNS process leading to drug addiction [2]. OT is usually administered as Intravenous (IV) infusion at a range of 0.02 units/mL (40 ng/mL) to 0.12 units/mL (240 ng/mL). Literature review suggests current analytical methods (HPLC-UV/LC-MS) have not established lower detection limits and easily reproducible transitions for the reliable quantitation and confirmation of OT in blood and pharmaceutical formulations (Pharmas).

Objective: Develop a more sensitive, reliable and rapid quantitative method to determine OT in blood and pharmas.

Method: Calibration standards were prepared in DI water. Blood and pharma samples containing OT were diluted with water; D-4 meperidine (blood) and Angiotensisn II (pharma) were used as internal standards. For blood sample analysis, negative blood with concentration below LLOQ were pooled and spiked with calibrators to create sample matrix. Samples were analyzed on Agilent 6460 quadrupole MSMS equipped with Infinity 1260 LC. Separation was achieved on C-18 column using gradient elution. Mobile phases of water:methanol (90:10 v/v) with 5mM ammonium formate (A) and acetonitrile with 0.1% formic acid (B) were used in gradient elution program; 30% B to 70% B over 2.5 min, returning to initial 30% B conditions over 0.5 min and held for 1 min for a total run time of 4 min. Data was acquired on MRM^+ mode and mass transitions (1007.3 -723.3/202.1/523.5 *m/z*) were observed. Surprisingly, the most intense peptide precursor is the singly charged species at 1007.3 *m/z* and not the expected double protonated ion at 504.2 *m/z*, which may be due to the fact that OT contains a disulfide bond that restricts peptide protonation.

Result: Calibration range of this method was shown to be linear (R^2 - 0.9969) from 0.005 U/mL (10 ng/mL) to 0.1 U/mL (200 ng/mL). LOD and LOQ were 0.0025 U/mL (5 ng/mL) and 0.005 U/mL respectively. Analytical recovery (82-84%) and % CV for intra & inter assay (n=4) were within the acceptable ranges. No significant carry over (< 2%) was observed at 0.1 U/mL.

Conclusion: We have developed a precise, sensitive and reproducible LCMSMS method for the determination of Oxytocin in blood and pharmaceutical formulations.

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Keywords: Oxytocin, LCMSMS

P51

Tizanidine Intoxication in a Postmortem Case

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Introduction: Tizanidine (Sirdalud, Ternelin, Zanaflex) is a synthetic alpha (2)-adrenergic antagonist, structurally related to clonidine, that has been used as an antispastic agent since 1975. Adverse effects attributed to tizanidine therapy include dizziness, sedation, asthenia and dry mouth. There is limited published data about deaths involving tizanidine intoxication.

Objective: This case presents a death associated with tizanidine intoxication.

Method: Postmortem femoral and heart blood, vitreous fluid, gastric, urine, liver and brain tissue were submitted for toxicological analysis. Analysis of the postmortem femoral blood utilized gas-chromatography/flame ionization detection (GC/FID) for volatile testing (methanol, ethanol, isopropanol and acetone) and enzyme multiplied immunoassay technique (EMIT) and liquid-liquid alkaline extraction followed by gas-chromatography/mass spectrometry (GC/MS) for blood drug screening. Femoral blood was sent to an external laboratory for quantitations on tizanidine (LC-MS/MS) and doxepin (GC).

Result: The decedent is a 55 year old female that was found unresponsive by her husband. The subject was worked on by paramedics for 40 minutes on the scene. She was transported to a hospital where she was pronounced dead seven minutes after admission. The subject's primary care physician stated that she was very depressed and had missed several appointments. She had a history of fibromyalgia, gastric bypass, bipolar disease and suicidal ideations. Toxicology screening indicated the presence of diphenhydramine and doxepin. No volatiles were detected. Doxepin was present at a level of 0.054mg/L in femoral blood. Diphenhydramine was not quantified. At the scene, an empty bottle of tizanidine was discovered. It had been prescribed 15 days prior to the subject's death, yet the 90 day supply was empty. Given these circumstances, tizanidine testing was requested and subsequently quantified at a level of 1mg/L in femoral blood.

Discussion: A previously published tizanidine postmortem case mentioned in Baselt(2014) determined toxic levels of tizanidine, ethanol and diazepam in a 57 year old female decedent. Whereas in that case the toxic levels of all drugs could be considered contributing factors to the subject's death, the tizanidine case presented here is unique because tizanidine is the only drug present in toxic levels in the decedent. Although other drugs are present, they exist in therapeutic levels; thus pointing to tizanidine as the major contributor to death in the subject.

Conclusion: Fatalities resulting from tizanidine overdose are very rare. Blood concentrations of tizanidine following therapeutic use generally do not exceed 0.025mg/L, yet the subject in this case had a tizanidine femoral blood level of 1mg/L. The cause of death as determined by the medical examiner was acute tizanidine intoxication. The manner of death was suicide.

Keywords: Tizanidine, Postmortem, Blood

P52
Cost/Benefit Analysis of Case Management Policies in a DUI Lab

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Introduction: Most laboratories conducting testing on antemortem specimens submitted for driving under the influence (DUI) cases have a protocol for the management of services performed on each case. This protocol usually involves limiting the number of blood drug screens (BDS) performed based on the blood alcohol concentration (BAC). Prior to September 2014 at Palm Beach County Sheriff's Office (PBSO) Toxicology Lab, there were two criteria that a case had to meet before a BDS was conducted. First, there had to be a request. Second, if the case did not involve a fatality, the ethanol concentration had to be less than 0.10 g/dL. It has been reported that by employing such a protocol, the number of drugs involved in DUI cases is vastly underreported. Beginning in September 2014, the PBSO BDS protocol was modified to enable further study on the impact on drugs reported due to a BDS protocol.

Objective: A study was conducted to evaluate the magnitude of the underreporting of drugs in DUI cases by using a BDS case management protocol and to determine whether not reporting those drugs would have a meaningful impact on the DUI cases. This work was published and a follow-up study was conducted to gather a larger data set for evaluation and compare the results to the original study.

Method: In the original study, a BAC and BDS was performed on 54 cases regardless of the officer's request, the severity of the case, or the BAC. Of those cases with positive drug results, they were considered to be significant for the purposes of this study if the ethanol level was below 0.15 g/dL and the concentration of the drugs were at or above the estimated therapeutic range for the drug (or a suitably high level for illicit compounds). For the follow-up study of 105 cases, the BDS protocol was modified so that a BDS was performed for all cases with a BAC less than 0.15 g/dL.

Result: In the original study, although drug positivity was high, a corresponding high number of meaningful drug results were only observed when the BAC was less than 0.08 g/dL.
For the follow-up study, the percentage of cases with meaningful drug results was almost identical to the original study. For those cases with a BAC < 0.08, ≥ 0.08, and ≥ 0.10 g/dL there were meaningful drug results in 83%, 6%, and 4%, respectively.

As part of the follow-up study on the BDS protocol, a cost analysis estimate was conducted using purchasing and statistical data for calendar year 2014. It was estimated that on average a BDS had a materials cost 30 times greater than a BAC and required over six times as much analyst time. To perform a BDS on every case as has been recommended, the estimated increase in analysis materials, cost and analyst time was 118% and 93%, respectively.

Conclusion: The results of this study futher support the insufficiency of presenting drug positivity as a justification for completing drug analysis on every DUI case. For the vast majority of cases with a BAC greater than 0.08 g/dL, the drugs detected are not significant for supporting a DUI charge and do not warrant the substantial increase in analysis cost and time required. Additional research should be conducted with quantitative drug results and casework impact of blood drug screen protocols.

Keywords: DUI/DWI, Drugs, Case Management

P53

Emerging Novel Psychoactive Substances: A Driving Under the Influence of Drugs Case Involving Methiopropamine

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Introduction: Over the past decade the recreational use of research drugs, referred to as novel psychoactive substances (NPS), has been a growing area of forensic toxicological analysis. In 2011 abuse of Methioproamine (1-(thiophen-2-yl)-2-methylaminopropane) (MPA), a thiopene ring-based structural analogue of methamphetamine, was first reported. Marketing of MPA as a "legal high" that is readily available over the internet has resulted in multiple reports of deaths and intoxications worldwide. Publications concerning MPA effects are limited, but drug user forums generally describe the effects as similar to other stimulant recreational drugs.

Objective: Successfully analyze biological specimens, including blood and urine, for the qualitative identification of methiopropamine.

Method: A 1 mg/mL methiopropamine standard was purchased from Cerilliant. The laboratory's current procedure for analysis of amphetamines in blood and urine, by liquid-liquid extraction, was performed and then analyzed via GC/MS. Relative retention time and mass spectra were obtain for the underivatized MPA standard, as well as acetic anhydride (acetyl) and (S)-(-)-N-(trifluoroacetyl)-prolyl chloride (TPC) derivatives. Additionally the possibility of interference with the analysis of other amphetamine type drugs, detection of MPA in blood using LC/MS/MS, and its cross reactivity with CEDIA Amphetamine/Esctasy assay in urine were examined.

Result: In 2014, a driving under the influence of drugs (DUID) case involving a 57 year old white male was submitted to the GBI for analysis. The subject was observed driving erratically and exhibited multiple clues during field sobriety tests. Initial screening of blood via LC/MS/MS was negative and CEDIA amphetamine/ecstasy assay in urine was indicative for this case. Standard urine amphetamine confirmation testing resulted in the discovery of an unknown analyte, later identified as methiopropamine. The MPA standard data was compared to the initial case data and subsequent confirmation testing was conducted. MPA was qualitatively identified in both the blood and urine specimens via the acetyl derivative and the urine sample was additionally confirmed in its underivatized and TPC derivative forms. MPA was determined to be suitable for future LC/MS/MS analysis. Cross reactivity with the urine CEDIA amphetamine/ecstasy assay is approximately 30%. When analyzed along with other commonly encountered amphetamine type drugs, methiopropamine exhibits similarities with methamphetamine in both mass spectra and relative retention time, but can be distinguished by the presence of the 97 and 124 ion clusters.

Conclusion: This was the first methiopropamine case reported in the state of Georgia. The subject in this case also tested positive for 23 µg/L of etizolam. According to drug user forums and European reports, poly drug use with MPA appears to be common, which makes attributing observed effects and behaviors specifically to MPA difficult. The subject was observed to be "out of it", having problems performing simple requests and demonstrating issues with balance. Field sobriety tests indicated body sway, body tremors, elevated pulse, dilated pupils with little reaction to light, lack of smooth pursuit, as well as both horizontal and vertical nystagmus. Identification and quantitation of methamphetamine in the presence of MPA is possible, due to differences in relative retention time and existence of unique ion clusters. Howerever, TPC derivatization and d vs l isomer determination of methamphetamine in urine may be effected due to coelution with MPA, if both analytes are present. Additional method validation is required to determine the possible addition of MPA to the laboratory's current LC/MS/MS screening method, as well as GC/MS quantitation of the drug.

Keywords: Methiopropamine (MPA), Novel Psychoactive Substances (NPS), Driving Under the Influence of Drugs (DUID)

P54
Extraction of Cannabinoids in Marijuana, Oil and Edibles by QuEChERS

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Introduction: Medical marijuana has been legalized in multiple states across the USA. As a result, many labs are seeking fast and reliable analytical methods to determine the cannabinoid potency in marijuana, oil and cannabis infused foods (also known as edibles). This application utilizes the advantages of the QuEChERS followed by either a dilution for marijuana samples or solid phase extraction (SPE) cleanup for complex food samples.

Objective: To develop a high throughput method to determine the cannabinoid potency in marijuana, oil and cannabis infused foods using GC-MS combined with QuEChERS for sample clean up and concentration.

Method: 1g of homogenized food samples (or 100 mg marijuana) were placed into 50-mL centrifuge tubes. 10 mL of reagent water was added to the tubes and the samples were allowed to soak for 30 minutes shaking occasionally. 10 mL of MeCN was added and samples were shaken for 1 minute (10 min for marijuana). EN15662 extraction salts were added to the samples, which were then shaken for 1 minute using a Spex 2010 Geno-Grinder at 1000 strokes/min. Samples were centrifuged at 3000 g for 5 minutes. Marijuana samples were prepared by mixing 5 µL of the supernatant with 1 mL n-hexane. Food samples were prepared by transferring 1mL of the supernatant to clean test tubes and were diluted 10x with pH 7 phosphate buffer. The buffered samples were added to SPE columns previously conditioned with 3 mL 1:1 n-hexane:ethyl acetate, followed by 3 mL methanol and 3 mL reagent water. Columns were washed with hexane and dried. Analytes were eluted with 3 x 1.5 mL of 1:1 n-hexane:ethyl acetate. The eluent was evaporated to dryness and reconstituted in n-hexane for GC/MS analysis.

Result: Matrix effect was determined by comparing the calibration curve slopes of the matrix matched standards against calibration standards prepared in n-hexane, which were found to be ≤ 20% for bread samples, and 45 to 60% for oil samples, thus calibration standards were prepared either in matrix or in solvent depending on the matrix effect of the particular sample tested. For both calibrations, 6-point calibration curves were constructed, the responses were linear with correlation coefficient greater than 0.995. Excellent accuracy and CV% were obtained (88.9 -113.4% with CV% ≤ 8.2% for bread, and 96.7 – 105.1% with CV% ≤ 7.4% for oil).

Discussion: Tetrahydrocannabinol, cannabidiol, and cannabinol were selected for analysis in this study. The method was applied to 6 seized marijuana samples, and the cannabinoid contents and phenotypic indexes were reported. Edible samples were assessed for cannabinoid potency levels. Combining a QuEChERS extraction with SPE cleanup allowed for the removal of food interfering compounds. Proteins were precipitated by acetonitrile in the QuEChERS extraction step, while the majority of the hydrophilic compounds, such as carbohydrates remained in the aqueous phase. Fatty and organic acids were retained onto the weak anion exchanging sorbent in the CSTHC column, however, they weren't eluted by the non-polar elution solvent.

The decision to utilize an external calibration was based on the high concentrations of the analytes of interest (ug or mg levels) as opposed to traditional forensic analysis (ng levels). The high concentrations will allow testing labs to use less sensitive and selective instruments, such as the GC-FID or HPLC-UV. These instruments can't separate the parent compounds from the deuterated IS preventing effective quantitation. The analytical performance was not comprised using external calibration in this study (RSD% < 5% and recovery 90-100).

Keywords: QuEChERS, Cannabinoids, SPE

P55
Seventeen Cases Involving Alpha-Pyrrolidinovalerophenone (α-PVP)

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Introduction: Seventeen cases involving α-PVP were submitted between 2012 and 2015 to the Western Department of Forensic Science (DFS) Lab. Fourteen suspected impaired driving cases were determined to have α-PVP concentrations less than 0.005 to 0.09 mg/L. Three fatalities during this period were determined to have α-PVP concentrations ranging from 0.03 to greater than 20 mg/L. Human use of synthetic cathinones like α-PVP have been reported to induce psychological effects like delusions, paranoia, hallucinations and deleterious cardiovascular effects.

Objective: The purpose of this presentation is to discuss cases involving α-PVP and potential physiological and psychological effects with α-PVP alone or in combination with other drugs.

Method: Internal standard (Amphetamine-D11, 100 µL) was added to 1 mL of blood, vortexed; followed by saturated trisodium phosphate buffer and chlorobutane. The samples were then rotated and centrifuged. Before drying samples with nitrogen at 40°C, 100 µL of 0.2% of hydrochloric acid in 2-propanol was added. Samples were reconstituted with 0.1% formic acid in water and injected on an Agilent 6430 triple quad with an Agilent Poroshell 120 EC-C18, 2.1x75mm, 2.7 µM particle size column. Mobile phase A consisted of 0.1% formic acid in water and mobile phase B consisted of 0.1% formic acid in acetonitrile. The MRM transitions were: α-PVP: m/z (232.2→126.1, 232.2→91); Amphetamine-D11: m/z (147.2→130.1, 147.2→98.1) in positive mode.

Result: The reported behaviors in the fourteen suspected impaired driving cases ranged from central nervous system depression to eluding officers in a high speed chase. The mean and median DUID α-PVP concentrations were 0.029 and 0.023 mg/L, respectively. Along with α-PVP, the following drugs were also commonly detected: benzodiazepines, oxycodone, tramadol, and cannabinoids. Buprenorphine was also commonly listed in the case histories; however, currently DFS does not screen for buprenorphine in suspected impaired driving cases. The α-PVP concentrations in the three fatalities were determined to be 0.033, 0.054, and present greater than 20 mg/L.

Conclusion: In fourteen DUID cases, only one case reported side effects consistent with synthetic cathinones. Two of the three fatalities indicated histories of bath salt and/or recreational drug use. At this time, no correlation can be determined between side effects and α-PVP concentrations.

Keywords: Alpha-Pyrrolidinovalerophenone (α-PVP), Impaired Driving, Postmortem

P56 ***"NIJ Funded"***

Involvement of Proton Pump Inhibitor Metabolites in the Inhibition of Buprenorphine Metabolism

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Introduction: We are performing ongoing studies on in vitro inhibition of opioids by different classes of potential drug interactants. One noted set of interactants is the proton pump inhibitors (PPIs). We have previously shown that PPIs cause preincubation time-dependent inhibition (TDI) of buprenorphine metabolism in human liver microsomes (HLM). Only rabeprazole, however, displays preincubation time and concentration dependent loss of specific cytochrome P450 (CYP) activity in a dual incubation system, the hallmark of mechanistic dependent inhibition (MDI). We propose that metabolites of the PPIs may contribute to the in vitro inhibition of buprenorphine metabolism.

Objective: To determine IC_{50} values for inhibition of CYP 3A4 dependent metabolism of buprenorphine by parent PPIs and their commercially available metabolites.

Method: Recombinant CYP3A4 was incubated with inhibitor at six concentrations (0.3 to 200 μM) along with a zero inhibitor control. Incubations were performed with or without a 15-minute preincubation of the CYP with inhibitor and a source of NADPH. Each incubation was performed in duplicate and repeated for an N=4 per concentration. Results were calculated as % control activity and plots were analyzed for IC_{50} determination and differences between ± preincubation using Prism GraphPad (v 5.0) software.

Result: Results to-date are summarized in the following table:

Inhibitor	IC_{50} (μM)		p-value
	- Preincubation	+ Preincubation	
Esomeprazole	158	26.3	0.0012
Omeprazole	123	22.8	0.0414
5-OH-Omeprazole	> 200	95.5	ND
Omeprazole Sulfone	200	> 200	NS
O-Desmethylomeprazole	91.2	3.82	< 0.0001
Lansoprazole	> 200	100	NS
5-OH-Lansoprazole	50.9	9.05	< 0.0001
Pantoprazole	> 200	> 200	NS
O-Desmethylpantoprazole Sulfone	29.4	64.6	NS
Rabeprazole	120	8.63	0.0475
Rabeprazole Sulfide	> 200	> 200	NS

Work continues to identify additional commercially available PPI metabolites and to assess their effect on buprenorphine metabolism by CYP2C8.

Conclusion/Discussion: We have shown that some metabolites of PPIs are as potent, and in some cases even more potent, inhibitors of CYP3A4 metabolism of buprenorphine. Inhibition by the metabolites may, or may not be preincubation time dependent. These findings help explain how TDI is observed in HLM, but are not MDIs of CYP3A4. Contribution of inhibitor metabolites to drug-drug interactions is a growing concern. Here we have demonstrated such a potential for PPI metabolites to interact with a widely used and often abused opioid.

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Keywords: Buprenorphine, Drug Interactions, Proton Pump Inhibitors

P57

Comparison of On-Site and Laboratory Based Techniques for the Analysis of THC in Oral Fluid Following a Single Smoking Session

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Introduction: Law enforcement departments in several states are evaluating rapid screening technology for oral fluid analysis in drivers. While there are many on-site tests available for oral fluid analysis, few are instrumented and allow for printing or retention of the result.

Objective: In this study, the objective was to determine whether an oral fluid rapid screening test could identify a single session of smoked marijuana and to compare the results to oral fluid laboratory analysis using both screening and confirmation.

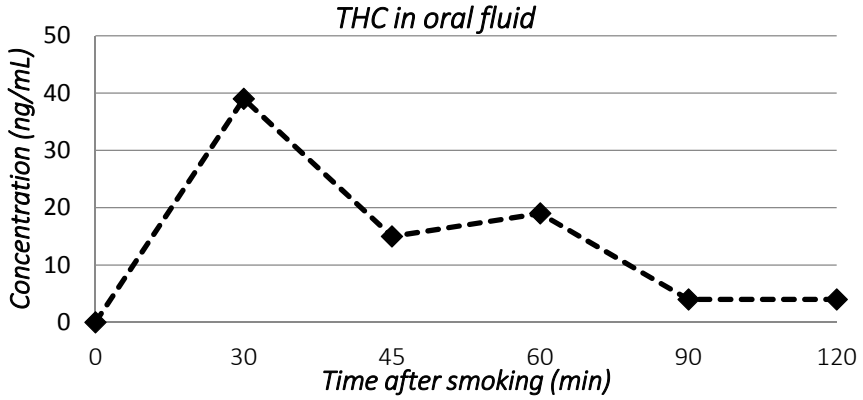
Method: Oral fluid specimens were collected from an adult male, a recreational infrequent user, who voluntarily smoked marijuana. The samples were analyzed using rapid screening technology (DDS®2, Alere; THC Cut-off: 25ng/mL), laboratory based immunoassay (ELISA) screening for THC (Cut-off: 4ng/mL; Immunalysis Corporation); and GC/MS for THC confirmation (LOQ: 1ng/mL). The subject smoked periodically for approximately 40 min; he reported feeling no further effects of marijuana about 2 hours after ending the smoking session. Samples were collected 30, 45, 60, 90, and 120 minutes after smoking. Confirmation specimens were collected using the Quantisal™ device at each time point.

Result:

DDS®2: The DDS®2 rapid screening device produced a negative result prior to smoking and positive results at time points 30, 45 and 60 min. Oral fluid specimens taken at time points 90 and 120 min were negative on the rapid test device.

ELISA: All Quantisal™ collected oral fluid specimens were positive using ELISA screening except for the sample at time zero (t_o) taken prior to starting the session.

GC/MS: THC concentrations established from Quantisal™ collected oral fluid specimens at the different time points are shown in the graph; samples were positive at all time-points except t_o.



Discussion: Oral fluid specimens tested on the DDS®2 were positive for THC for at least 60 min after an occasional marijuana user smoked a relatively low amount of cannabis in a recreational setting. Laboratory based testing showed the samples to be positive up to 2 hours using ELISA and GC/MS confirmation. The GC/MS concentrations were lower than 25ng/mL after 45 min, but the DDS®2 device still provided a positive result after 45 and 60 minutes. It is likely other cannabinoids in the oral mucosa contributed to a positive screen. Even though the information is from only one subject, data from other recently published studies using oral fluid collected by four different devices showed peak THC levels 15 – 30 minutes after smoking and THC concentrations above 10ng/mL after one hour.

Summary: Oral fluid analysis from a rapid screening device (DDS®2) correlated well with established laboratory based testing; data supports the utility of the DDS®2 to detect recent marijuana use (up to 60 min) from a single smoking session, and is in agreement with other published studies.

Keywords: Rapid Tests, Marijuana, Oral Fluid

P58

Extended Cannabinoid Stability in Authentic Oral Fluid After Controlled Cannabis Smoking

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Introduction: Oral fluid drug testing is becoming more common; therefore, oral fluid drug stability is an important consideration for assuring accurate results. We previously established oral fluid stability of Δ9-tetrahydrocannabinol (THC), 11-nor-9-carboxy-THC (THCCOOH), cannabidiol (CBD), and cannabinol (CBN) in authentic oral fluid collected with Quantisal™, Oral-Eze®, and StatSure™ devices stored at 4°C for up to 4 weeks. Extended refrigerated stability (>4 weeks) would be helpful for clinical and forensic testing and court-mandated reanalysis.

Objective: To examine extended cannabinoid stability by evaluating THC, THCCOOH, CBD, cannabigerol (CBG), and tetrahydrocannabivarin (THCV) concentrations in pooled authentic oral fluid stored at 4°C for up to 3 months.

Method: Six healthy 18-50 year old cannabis smokers provided written informed consent for this Institutional Review Board-approved study. A pool of oral fluid collected with Quantisal™ devices was prepared for each participant following smoking of a 6.9% THC cigarette; specimens collected at -1.5, 1.5, 3.5, 5, 8, 10, 12 and 14 h were mixed to create participant stability pools. Duplicate specimens were stored at 4°C for one day (baseline), and one, two and three months before analysis. Following red abalone β-glucuronidase hydrolysis and subsequent solid-phase extraction, cannabinoids were quantified by a previously validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method using atmospheric pressure chemical ionization (APCI) with multiple reaction monitoring. Limits of quantification (LOQ) were 15 ng/L THCCOOH and 0.2 µg/L for all other analytes; inter-assay accuracy and imprecision were 88.1-105.5% and 5.8-8.2% coefficient of variation, respectively (n=92). Results within ±20% of baseline concentrations were considered stable.

Result: Baseline CBD, CBG, THCV and THCCOOH concentrations did not exceed LOQ in 3 of 6, 2 of 6, 4 of 6 and 3 of 6 participants, respectively. Median (range) baseline concentrations were THC 6.7 (3.8-39.2, n=6), CBD 0.8 (0.2-1.6, n=3), CBG 0.7 (0.3-1.4, n=4) and THCV 0.5 (0.5-0.6, n=2) µg/L; median (range) THCCOOH baseline concentrations were 0.12 ng/L (0.03-0.20, n=3). All analytes were stable for up to two months at 4°C for all participants with positive baseline analyte concentrations. THC, CBD, CBG, THCV and THCCOOH were stable for up to three months for 5 of 6, 2 of 3, 3 of 4, 1 of 2 and 2 of 3 participants, respectively. Mean concentrations for the remaining participants decreased 21, 22, 23, 23 and 27% after three months at 4°C for THC, CBD, CBG, THCV and THCCOOH, respectively.

Conclusion: Quantisal-collected oral fluid specimens should be stored at 4°C for no more than two months to assure accurate THC, CBD, CBG, THCV and THCCOOH quantitative results.

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

Keywords: Cannabinoids, Oral Fluid, Stability

P59

Determination of ETS Exposure in Pet Dogs Using Optimised Methanol Extraction of Fur Followed by Hydrophilic Interaction Chromatography - Tandem Mass Spectrometry

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Introduction: Nicotine is a highly toxic alkaloid that causes stimulation of autonomic ganglia and the central nervous system. Measurement of nicotine in hair can be an informative tool for the assessment of long-term environmental tobacco smoke (ETS) exposure and related health problems. Collection and analysis of pet dog fur for the presence of nicotine and its metabolite cotinine provides an insight into ETS exposure in pet dogs and cancer risk to both dogs and humans.

Objective: Two fur samples were collected from 15 pet dogs approximately 12 months apart, and analysed for the presence of nicotine and its main metabolite cotinine to evaluate changes in ETS exposure in dogs. In addition, methanolic wash efficiency and optimisation of the extraction incubation period were investigated.

Method: Fur samples (N=30) were first homogenised by cutting each sample separately into 1-2 cm lengths. From each sample, 30 mg of fur was washed with 2 mL of methanol by sonicating for 5 minutes. The methanol wash was then transferred to a clean vial and spiked with deuterated nicotine and cotinine internal standards (1 ng/mg). The fur samples were dried in an oven at 40°C for 30 minutes and then cut into 1-2 mm snippets. Methanolic extraction was achieved by incubating fur samples in 2 mL of methanol with 0.1% formic acid and 200 µl aliquot analysed after 15, 30, 60 and 120 minutes of sonication.
An Agilent LC–MS-MS triple quadruple G6410B mass spectrometer equipped with an Agilent 1200 series auto-sampler utilising electrospray ionization was operated in multiple reaction monitoring (MRM) mode. The column used was a SeQuant® ZIC®-HILIC (5µm,200Å) PEEK 150 x 4.6 mm coupled with a C18 guard column (42.0 mm). As organic solvents are weak solvents in HILIC mode, the methanol extract and wash were injected straight onto the HILIC column, minimising nicotine loss. Nicotine and cotinine were linear over the range 0.01 – 10 ng/mg with correlation coefficients of $R^2 \geq 0.99$.

Result: Nicotine was detected in all methanol wash samples although only 12 were above the lower limit of quantitation (LLOQ) (10 pg/mg) at concentrations ranging from 0.02 to 0.31 ng/mg (mean = 0.11, median 0.095). Cotinine was detected in 50% of the methanol wash samples and above the LLOQ in 10 samples at concentrations ranging from 0.04 to 5.9 ng/mg (mean = 1.06, median 0.53).
The recovery of nicotine from fur was assessed over a 2 hour sonication period and reached a plateau between 1-2 hours. In contrast, cotinine reached a plateau within 30 minutes and then the recovery decreased significantly with increasing incubation time. Nicotine and cotinine concentrations in washed fur samples ranged from 0.02 to 4.1 and 0.01 to 2.7 ng/mg, respectively.

Discussion: The presence of cotinine in the methanol wash may indicate extraction of cotinine from inside the fur but could also be on the outer surface due to formation following tobacco smoking or via atmospheric oxidation of nicotine. The mean nicotine to cotinine ratio for the methanol wash was 0.48 compared with 12.78 for the fur samples and calculation of the ratio may be a suitable approach for assessing endogenous versus exogenous nicotine exposure in dogs.

Keywords: Nicotine/Cotinine, Dog Fur, HILIC

P60

Validation of a Method for Analysis of Buprenorphine, Norbuprenorphine, and Naloxone in Blood Using LCMSMS

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Introduction: Buprenorphine is a synthetic partial mu- opioid agonist typically used in pain management and opioid withdrawal clinics. Buprenorphine has been available in the US since 1985 and has become increasingly more popular than methadone in the treatment of opioid dependent patients. Buprenorphine has analgesic properties that are up to 40 times as potent as morphine and, as a CNS depressant, can cause drowsiness, dizziness and confusion. Because of its increasing popularity and potential for abuse, forensic toxicology laboratories should be able detect and quantitate buprenorphine and its major active metabolite, norbuprenorphine. Buprenorphine is available in a variety of formulations including films, sublingual tablets, transdermal patches and injection solutions. Some formulations contain buprenorphine only and others contain buprenorphine and the antagonist naloxone.

Objective: The objective of this work was to develop and validate a liquid chromatography tandem mass spectrometry (LCMSMS) method for the quantitation of buprenorphine, norbuprenorphine, and naloxone in postmortem and antemortem blood following SWGTOX method validation guidelines.

Method: A solid phase extraction (SPE) method with an Agilent 1200 Infinity series High Performance Liquid Chromatography (HPLC) system combined with an Agilent 6430 Triple Quadrupole LCMS system was developed and validated to quantitate buprenorphine, norbuprenorphine, and naloxone. Deuterated internal standards and 2 mL pH 6.0 phosphate buffer were added to 2 mL of sample. After vortexing, the samples were centrifuged and applied to UCT Cleanscreen© SPE columns conditioned with water, methanol, and buffer. The columns were then washed with water, 1N acetic acid and methanol before elution with dichlormethane:2-propanol:ammonium hydroxide (80:20:2). Samples were reconstituted in mobile phase A (LCMS grade water with 0.1% formic acid) and injected onto the LCMSMS system. HPLC separation was achieved using an Agilent Poroshell 120 EC C18 column (2.7 µm x 2.1 x 75mm) and gradient elution at 40°C. The method was validated following SWGTOX guidelines for accuracy, bias and precision, limits of detection and quantitation, calibration model, selectivity/specificity, carryover, matrix effects/ion suppression, dilution integrity and stability.

Result: Between run and within run accuracy and bias/precision were determined not to exceed CV values of +/- 20% for all compounds. Between run mean accuracy ranged from 93.1% - 107%. Between run precision was within 15% for all compounds (range 5.3% - 13.6%.) The limit of detection was determined to be 0.10 ng/mL, 0.25 ng/mL and 0.10 ng/mL for buprenorphine, norbuprenorphine and naloxone. The lower limit of quantitation was determined to be 1.0 ng/mL for buprenorphine and 0.50 ng/mL for norbuprenorphine and naloxone. The upper limit of quantitation was 20 ng/mL, with a linear 1/x weighted calibration model, for all compounds. No significant interference from matrix effects or common drugs of abuse was observed. Recovery was greater than 75% for all compounds at high and low concentrations.

Conclusion: A method for confirmation and quantitation of buprenorphine, norbuprenorphine, and naloxone in blood was successfully developed and validated using SPE and LCMSMS. Since Alabama Department of Forensic Sciences (ADFS) began screening for buprenorphine with EIA, over 50 cases have been confirmed. This new method allows ADFS to conduct the confirmation testing in-house rather than submitting cases to a reference laboratory. This is one of the first published methods to quantitate naloxone along with buprenorphine/norbuprenorphine in blood. The ability to detect naloxone can help distinguish between different formulations of buprenorphine such as Suboxone® and Butrans®.

Keywords: Buprenorphine, LCMSMS, Validation

P61
Molly Mosquito Detection in Two Cases of Designer Ecstasy Use

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Introduction: Substituted amphetamines are commonly used and abused for their entactogen effects. Currently, these drugs are halogen substituted amphetamines. With the substitution of a fluorine molecule on the aromatic ring, fluoroamphetamine becomes more lipophilic than amphetamine and thus can more easily cross the blood-brain barrier. 4-Fluoroamphetamaine is the most commonly detected substituted amphetamine, and it goes by the street name "Molly Mosquito". It has similar entactogen effects as MDMA (Molly) and is currently not scheduled by the US Drug Enforcement Agency.

Case 1: An 18 year old female presented to the emergency department (ED) after drinking two capfuls of "Molly's Mosquito cap" which she believed was a "synthetic ecstasy." After the initial euphoric effects subsided, she experienced headache, nausea, vomiting, lightheadedness, and diaphoresis. On arrival to the ED, her only complaint was anxiety. An echocardiogram obtained approximately 36 hours after ingestion revealed a cardiac ejection fraction of 10-15% with mild left ventricular dilation and severe diffuse hypokinesis. Forty-eight hours after admission she remained mildly tachycardic though her symptoms had improved and she was discharged.

Case 2: A 27-year-old man with polysubstance dependence presented to the ED after being found agitated and lying in the street. In the ED, His heart rate was 156 bpm and his rectal temperature was 106.5°F. He was agitated with non-sensible speech, diaphoresis, dilated pupils, and hyperreflexia without clonus. Urine toxicology was positive for amphetamines and phencyclidine (PCP) and negative for barbiturates, benzodiazepines, cocaine metabolite, methadone, opiates and tetrahydrocannabinol metabolite. On day 1, the patient admitted ingesting 200 mg of 4-fluoroamphetamine powder approximately four hours prior to ED presentation. On day 2, he left the hospital against medical advice.

Objective: To develop a method for the qualification and quantification of the fluoroamphetamine isomers, 2-fluoroamphetamine and 4-fluoramphetamine in biological specimens.

Method: Specimens were extracted by a previously published amphetamine method. In brief, a seven point calibration curve ranged from 50-5000 ng/mL and quality control samples (50, 150, 300, 4000, and 10000 ng/ml) were analyzed with each batch. Fluoroamphetamines were extracted from the specimen using ammonium hydroxide and n-butyl chloride. To the n-butyl chloride, 0.1 ml 1% HCL in methanol was added. It was then evaporated to dryness under a gentle stream of nitrogen at room temperature and the residue was derivatized using heptafluorobutyric anhydride, (HFBA). Analysis was performed using a Shimadzu gas chromatography mass spectrometry, QP-2010, with EI ionization (Shimadzu Scientific Inc., Columbia, MD). PCP analysis was performed as part of routine laboratory work.

Result: Case 1, urine was the only specimen received from the 18 year old female. The 4-fluoramphetamine urine concentration was 37,000 ng/ml. Four specimens were received from the 27 year old male. His admission urine contained 285,000 ng/ml 4-Fluoramphetamine and 107 ng/ml PCP. Specimens from seven hour post-admission contained the following: urine (124,000 ng/ml 4-Fluoramphetamine and 12 ng/ml PCP), serum (1400 ng/ml 4-Fluoramphetamine and 4 ng/ml PCP) and plasma (1300 ng/ml 4-Fluoramphetamine and 4 ng/ml PCP). The observed r^2 values for the calibration curves were 0.997 or better. The LOQ was administratively set at 50 ng/mL Validation criteria for calibrators and quality control specimens as well as matrix effects, absolute recovery, carryover and specificity were acceptable. Amphetamine, methamphetamine, MDA, and MDMA at 10,000 ng/ml did not interfere with either fluoroamphetamine isomer determination.

Conclusion: Acute dilated cardiomyopathy occurred in a young female exposed to substituted amphetamine. Cardiac decompensation may occur in patients acutely intoxicated from amphetamines though these symptoms are short lived and reversible. The previously published amphetamine method is also valid for the analysis of 2 & 4-fluoroamphetamine as well as routinely analyzed amphetamines.

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Keywords: Molly Mosquito, 4-Fluoroamphetamine, GC-MS

P62

A Comparative Meta-Analysis of Tick Paralysis in North America and Australia: Epidemiology, Clinical and Electrodiagnostic Manifestations, and Outcomes

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Background: Tick paralysis (TP) is an unusual, potentially fatal, neurotoxic poisoning that mimics botulism, polio, and the Guillain-Barre Syndrome (GBS) and occurs in regional pockets of the United States (US) and Canadian Pacific Northwest and the Australian East Coast. TP is caused by the injection of uncharacterized neurotoxins during blood-feeding by gravid, ixodid- species, hard ticks, shortly before ovipositing. The neurotoxins probably block sodium channels and inhibit presynaptic release of acetylcholine as suggested by electrodiagnostic studies in man and animals (Grattan-Smith PJ, et al. *Brain*, 1997).

Objective: In order to compare the epidemiology, clinical and electrodiagnostic manifestations, and outcomes of TP in North America and Australia, Internet search engines were queried to identify peer-reviewed, published case reports and case series of TP.

Method: 52 published cases of TP in the US over the reporting period 1898-2010 and 15 published cases of TP in Australia over the reporting period 1900-2010 were identified by Internet search. Continuous data including age, time to tick removal, and duration of paralysis were compared for statistically significant differences by unpaired t-tests. Categorical, demographic variables including gender, geographic distributions, tick vectors, and misdiagnoses were compared for statistically significant differences by Fisher exact tests. Statistical significance was defined by p-values less than 0.05.

Result: The demographic characteristics of the 2 populations were similar as both were composed of young girls and adult males presenting during spring-summer seasons. In addition, tick attachment sites were located predominantly on the head, scalp, and ears in both North America and Australia. *Dermacentor andersoni* was the predominant vector in the US, and *Ixodes holocyclus* was the predominant vector in Australia. TP cases clustered in the Pacific Northwest and Rocky Mountain states in the US, and on the Southeast Coast of Australia. The case fatality rates (CFR) for TP in North America were higher during the reporting period 1898-1954 (11.7%) than during the later reporting period of 1946-1996 (3%). There were 20 reported TP deaths in Australia during the reporting period 1900-1945. The time to correct diagnosis was significantly longer in Australia ($p = 0.013$), and the time to unassisted ambulation was also significantly longer in Australia ($p = 0.0001$) indicating more intense paralytic neurotoxicity. TP cases were more likely to have been misdiagnosed as GBS syndrome in the US than in Australia.

Discussion/Conclusion: TP is a seasonally and regionally predictable paralysis of young children and adult males that has been increasingly misdiagnosed as GBS in the US (Vedanarayanan VV et al. *Neurology*, 2002). Misdiagnoses in these cases delayed correct diagnosis and proper treatment by tick removal, and often directed unnecessary, high-risk therapies, such as plasmapheresis with intravenous immunoglobulin G (IVIG). TP should be added to and quickly excluded from the differential diagnosis of acute ataxia with ascending flaccid paralysis, especially in persons living in TP-endemic regions, by carefully searching for attached, blood-feeding ixodid ticks which may be hidden on the scalp and behind the ears.

Keywords: Tick Paralysis, Guillain-Barre Syndrome

P63
Deaths from Propofol: Accidental and Intentional

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Background: Since its introduction in 1986, propofol (2, 6-diisopropylphenol), an intravenous sedative-hypnotic agent with an unknown mechanism of action, has been used for the induction and maintenance of general anesthesia and conscious sedation in over 80% of cases; largely replacing thiopental (Wilson C et al. *Clin Toxicol*, 2010). Unrestricted as a controlled substance, propofol's abuse potential emerged quickly and was highlighted by the death of pop singer, Michael Jackson, in 2009 (Wilson C et al. *Clin Toxicol*, 2010).

Objective: In order to assess the epidemiological features of fatal propofol abuse, a descriptive analysis of the scientific literature was conducted using Internet search engines, including Medline, PubMed, Ovid, Google Scholar, and Cochrane.

Method: Well-documented cases of fatal propofol abuse were stratified as unintentional or accidental deaths and as intentional deaths by suicides or homicides (Iwersen-Bergmann S et al. *Int J Legal Med*, 2001; Roussin A et al. *Br J Anaesth*, 2006; Kranioti EF et al. *Forensic Sci Int*, 2007; Klausz G et al *J Forensic Legal Med*, 2009; Kirby RR et al. *Anesth Analg*, 2009; Levy RJ J *Forensic Sci*, 2011). Demographic characteristics of decedents were described including age, gender, occupation, and propofol blood levels. Continuous variables were compared for differences by unpaired, two-tailed t-tests with statistical significance indicated by p-values < 0.05.

Result: Of 21 fatal cases of propofol abuse, 18 (86%) occurred in healthcare workers, mostly anesthesiologists and nurse anesthetists (n=14, 67%). One case occurred in a layman who purchased propofol on the Internet. 17 deaths (81%) were accidental; 2 were suicides (9.5%) and 2 were homicides (9.5%). Fatal cases in which postmortem propofol blood levels drawn from the heart or the femoral artery were reported (n=12, 57%) are compared in the Table. Blood levels in intentional death cases were significantly greater than in accidental death cases ($p < 0.0001$); all of which reflected initial therapeutic doses in the ranges of 2.0-2.5 mg/kg. When reported, ethanol and benzodiazepines (midazolam, diazepam) were the most commonly co-administered drugs in fatal propofol overdoses.

Deaths from propofol	# reporting blood levels	Age in years (mean ± SD)	# Males: # Females (ratios)	Blood levels in µg/mL (mean ± SD)
Accidental deaths	6	33 ± 9.6	4:2 (2:1)	1.52 ± 2.1
Suicides	2	33 ± 5.5	1:1 (1:1)	1.36 ± 1.6
Homicides	1	24	1 F	4.3
Intentional deaths = Suicides + Homicides	3	30 ± 6.6	1:2 (1:2)	2.34 ± 2.1
t-test values (unintentional v. intentional deaths)	Not applicable (NA)	0.482	NA	6.93
p-values (unintentional v. intentional deaths)	NA	0.644	NA	< 0.0001

Discussion/Conclusion: In this study, fatalities from respiratory arrest occurred despite administration of therapeutic induction doses in accidental death cases among medical professionals compared to overdoses in suicides and homicides. Healthcare workers have much easier access to propofol than the general public. In a prevalence survey of one or more episodes of propofol abuse over a 10-year period in all126 US anesthesiology training programs, 29 cases with 6 deaths in 16 anesthesiology residents (38%) were reported (Wischmeyer PE et al. *Anesthesiology*, 2007). Surviving abusers included attending anesthesiologists (n=6), nurse anesthetists (n=3), anesthesia technicians (n=2), and operating room (OR) nurses (n=2) (Wischmeyer PE et al. *Anesthesiology*, 2007). The abuse of propofol by young healthcare professionals, particularly OR workers, was significant; and likely underreported (Wischmeyer PE et al. *Anesthesiology*, 2007). Propofol is a dangerous drug with an abuse potential that often results in fatalities. Propofol should be regulated as a controlled substance.

Keywords: Propofol, Diprivan®, Abuse, Addiction

P64 WITHDRAWN

P65 WITHDRAWN

P66

Concentrations of THC and Carboxy-THC in the Blood of Drivers and Postmortem Cases

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Introduction: The determination of tetrahydrocannabinol (THC) and its primary metabolite (Carboxy-THC) are high on the list of requested analytical tests performed by forensic toxicology laboratories. Interpretation of these drugs in post mortem samples is often based on the data from living subjects. 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).

Objective: This study was initiated with the idea of comparing the concentrations of THC and Carboxy-THC in post mortem blood samples with those found in drugs/driving cases when extracted and analyzed using the same protocol. The data will assist those analysts involved in the analysis and interpretation of THC and metabolite in both types of cases.

Method: Samples of blood (1 mL (Calibrators, controls, and test containing deuterated internal standards were precipitated with acetonitrile (2 mL)) which was removed and evaporated to 0.5 mL, and adjusted to pH 7 with 4 mL of an aqueous buffer, then extracted on mixed mode SPE columns. The SPE columns having being pre-conditioned with methanol and 0.1M phosphate buffer (3 mL, 3 mL respectively) prior to sample loading. The SPE cartridges were washed with pH 7 buffer (3 mL). Each SPE column was dried and eluted with 3 mL of a solution of ethyl acetate-hexanes-acetic acid (49-49-2). The samples were evaporated to dryness and the residues dissolved in 100 µL of methanol for LC-MS/MS analysis in positive/negative multiple reaction monitoring (MRM) mode for the respective analytes. Liquid chromatography was performed in gradient mode employing a 50 mm x 2.1 mm (2.1 µm) aromatic phase analytical column.

Result: The limits of detection/ quantification for this method were determined to be 0.5 ng/ mL and 1.0 ng/ mL, respectively for the analytes (THC and Carboxy-THC) The method was found to be linear from 1.0 ng/ mL to 100.0 ng/ mL ($r^2>0.999$).Recoveries were found to be greater than 95%. Interday and Intraday analysis of the analytes were found to < 8% and < 10 %, respectively. Matrix effects were < 6%.The results of post mortem samples involving 592 males, the THC range was 1 to 92 ng/ mL, Carboxy-THC range was 1-187 ng/ mL; for 396 females the THC range was 1 to 67 ng/ mL and Carboxy-THC range was 1 to 92 ng/mL. For drug/driving cases: in 201 males THC range was 1 to 29 ng/ mL, Carboxy-THC range was 1 to 186 ng/ mL, in 72 females THC ranged 1 to 17 ng/ mL, Carboxy-THC range was 1 to 65 ng/ mL.

Conclusion: This study shows that using a single extraction and analysis, cannabinoids from both types of cases can be evaluated. The interpretation of concentrations of THC and Carboxy-THC in post mortem samples relative to those obtained from living subjects should be treated with caution due to the wider range of concentrations found in post mortem samples.

Keywords: Cannabinoids, SPE, Blood

P67

Measurement Uncertainty for Vaporous Ethanol Concentration Analyzed by Intoxilyzer® 8000 Instruments

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Background: Breath Alcohol Analysis is used by law enforcement agencies to determine the alcohol concentration of individuals suspected of impaired driving. These concentration measurements have a finite and measurable amount of associated uncertainty characterized by the dispersion of values attributed to these measurements. This laboratory has conducted a study to estimate a reasonable measurement of uncertainty for vaporous ethanol as determined by the Intoxilyzer® 8000 evidential analyzer.

Objective: This analysis was performed to determine the measurement uncertainty associated with the calibration and function of the Intoxilyzer® 8000 manufactured by CMI, Inc. (Owensboro, KY) for the State of New Mexico.

The measurand is represented by the following formulas:

$$u_{prop} = \sqrt{Cal_{prop}^2 + A_{prop}^2 + CRM_{prop}^2 + IA_{prop}^2}$$

$$u_{const} = \sqrt{Cal_{const}^2 + IC_{const}^2 + IA_{const}^2}$$

$$u_c = \sqrt{u_{prop}^2 + u_{const}^2}$$

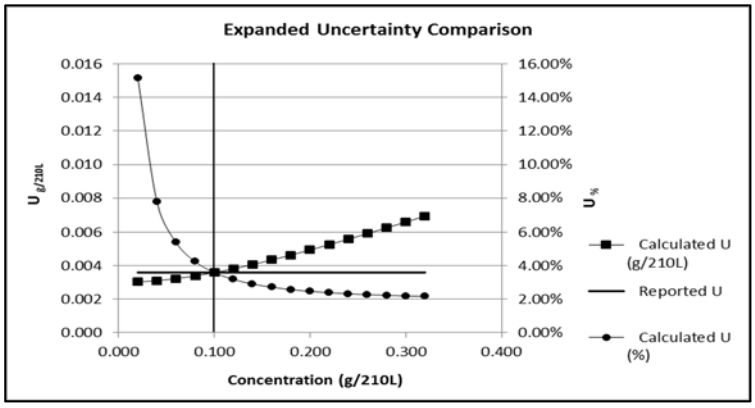
Where:

Cal	*: GC calibration adjustment*
A	*: GC analytical*
CRM	*: Certified Reference Material*
IC	*: Intoxilyzer® 8000 calibration adjustment*
IA	*: Intoxilyzer® 8000 analytical*
u_{prop}	*: combined proportional standard uncertainty*
u_{const}	*: combined constant standard uncertainty*
u_c	*: combined standard uncertainty*

Result: Five groups were identified as the major sources of uncertainty: GC calibration adjustment (Cal), GC analytical (A), certified reference material (CRM), Intoxilyzer® 8000 calibration adjustment (IC), Intoxilyzer® 8000 analytical (IA). Other sources were analyzed and considered to be inherent in the major sources of uncertainty.

A linear fit of response ratios, relative to n-propanol, was used for the calibration adjustment on the GC, which were performed in replicate to determine the slope and y-intercept variation of the method. Data for the GC analytical portion of uncertainty was collected from the historical data of QC samples and the analysis of the 0.080 and 0.160 g/210L wet-bath simulator solutions from the previous three years. The certified reference materials were treated as Type B uniform distribution.

The Intoxilyzer® 8000 calibration adjustment measurements were conducted on two instruments using a blank and five calibrator solutions. From each calibration set, a mean and a standard deviation (g/210L) were calculated and pooled to return a standard uncertainty. The Intoxilyzer® 8000 analytical uncertainty was assessed by plotting and analyzing in-field proficiency results; the slope and y-intercept were used as the proportional and constant standard uncertainties.



The standard uncertainties attributed to the previous sources were combined into a proportional standard uncertainty (0.9800%) and a constant standard uncertainty (0.0015 g/210L) using the method of root-sum-squares. Combining the standard uncertainties by category allowed for a simplified equation when estimating the standard uncertainty for the entire method.

Conclusion: A combination of reporting the expanded uncertainty was decided upon to reduce large discrepancies and to prevent any under reporting of the uncertainty. The expanded uncertainties are represented, in the graph above, both as a constant expanded uncertainty in g/210L and as a proportional expanded uncertainty in percentage compared to the reported uncertainty. This graph shows a comparison between the decided reported expanded uncertainty and the expanded uncertainty calculated as a percentage and a unit.

In the state of New Mexico, the following will appear on a subject breath test printout: A measurement of uncertainty for all BrAC at or above 0.10 g/210L is (+/-) 3.6%, and for all BrAC below 0.10 g/210L is (+/-) 0.0036 g/210L at a confidence level of 95%.

Keywords: Measurement of Uncertainty, Breath Alcohol, Intoxilyzer® 8000

P68

Development of a New Biochip Array for the Simultaneous Screening of the Recommended Drugs for DUID in Whole Blood

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Introduction: Drug impaired driving is becoming a major problem, recommendations for the toxicological investigation of drug-impaired driving and motor vehicle fatalities were reported. They focused on a two-tier approach of drug analysis. 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.[1] Tier 2 drugs being less frequently encountered. Biochip array technology enables the multi-analytical screening from a single sample.

Objective: This study aimed to develop a biochip array for the simultaneous screening of Tier 1 and Tier 2 drugs in whole blood for Driving Under the Influence of Drugs (DUID) testing.

Method: Twenty competitive chemiluminescent and semi-quantitative immunoassays arrayed on a biochip surface (15 Tier 1 assays and 5 Tier 2 assays), applicable to both the fully automated Evidence and the semi-automated Evidence Investigator analysers were employed. The sample volume required is 60µl of whole blood (diluted 1 in 4).

Tier 1 assays: 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: Buprenorphine (BUP), Dextromethorphan (DMP), Fentanyl (FENT), Tramadol (TRM), and Tricyclic antidepressants (TCAs).

Result: The cut-offs of all the assays were within the values stated in the recommendations. Limits of Detection (LOD) for Tier 1 assays: AMPH 4.35 ng/mL, MAMP 2.44ng/mL, BARB 4.41 ng/mL, BENZ1 0.26 ng/mL, BENZ2 0.83 ng/mL, THC 1.78 ng/mL, BZG 1.31 ng/mL, OPDS 1.37 ng/mL, MPB 13.15 ng/mL, MDONE 0.61 ng/mL, OPIAT 0.48 ng/mL, OXYC1 0.87 ng/mL, OXYC2 2.22 ng/mL, PCP 0.10 ng/mL, ZOL 0.45 ng/mL. LODs for Tier 2 assays: BUP 0.06 ng/mL, DMP 0.3 ng/mL, FENT 0.13 ng/mL, TRM 0.53 ng/mL, TCA 1.98 ng/mL. Intra-precision around the cut-off value for each of the assays, expressed as CV(%) (n=6), ranged between 5.5-16.3.

Conclusion: This new biochip array enables the simultaneous screening of drugs associated with DUID included under reported recommendation (Tier 1 and Tier 2). The LODs were lower than the recommended cut-offs in whole blood. This leads to test consolidation and increased screening capacity in test settings.

Reference: Logan, B.K. et al (2013); Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities; *Journal of Analytical Toxicology* 37(8):552-558.

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

P69

In vitro Metabolism Studies on Methylenedioxy-Substituted Amphetamines Using Human Liver Microsomes and LC/MS/MS with Chemical Derivatization

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Introduction: The abuse of β-keto derivatives of MDMA such as butylone and methylone has been reported since the mid-2000s. The forensic toxicologist faces problems with these drugs, because of lack of information on their metabolism and unavailability of reference standards of the metabolites and the parent drugs themselves. Ethical considerations usually preclude human metabolism studies under controlled conditions and *in vitro* methods provide potential alternatives. A few previous studies showed that β-keto derivatives of MDMA have similar metabolic pathways to MDMA, resulting in metabolites formed by demethylenation, demethylenation plus *O*-methylation (4-OH-3-MeO metabolites) and N-demethylation. Another challenge is the analysis of these polar metabolites using conventional C18 HPLC columns, resulting in low retention volumes. One rapid and simple solution to this is chemical derivatization. Conversion of metabolites to more hydrophobic compounds by derivatization can improve separations by reversed-phase HPLC and increase sensitivity in mass spectrometry.

Objective: To identify metabolites of butylone and methylone in humans using human liver microsomes (HLM) *in vitro* and reversed-phase LC/MS/MS with derivatization. MDMA was used in this study as a model compound as its metabolites are commercially available.

Method: Pooled human liver microsomes were incubated with NADPH regenerating system and MDMA, butylone or methylone in 0.1 M phosphate buffer pH 7.4 in a shaking incubator at 37°C for 90 minutes. Each reaction was stopped by the addition of ice-cold acetonitrile and extracts were derivatized with acetic anhydride/pyridine (3:2 v:v) for 30 minutes at 60°C. Derivatized metabolites were identified by LC/MS/MS using multiple reaction monitoring (MRM). Ten MDMA positive urines were also analysed to compare the metabolite patterns with those obtained *in vitro* with HLM.

Result: Three phase-I metabolites (both major and minor metabolites) of MDMA, butylone and methylone were detected. For MDMA, 3,4-dihydroxymethamphetamine derivative (HHMA-3Ac) was identified by MRM transitions at m/z 308>266, 308>224 and 308>151, 4-hydroxy-3-methoxymethamphetamine derivative (HMMA-2Ac) at m/z 280>238, 280>165 and 280>137 and MDA-Ac at m/z 222>163, 222>135 and 222>105. For methylone, dihydroxymethcathinone (DHMC), a major metabolite, was identified by MRM transitions at m/z 322>280 and 322>178 (DHMC-3Ac). Nor-methylone (a minor metabolite) was identified by MRM transitions at m/z 236>146 and 236>118 (bk-MDA-Ac). Another metabolite, 4-hydroxy-3-methoxymethcathinone (4-HMMC) was identified by MRM transitions at m/z 294>234 and 294>160 (4-HMMC-2Ac). Furthermore, a major metabolite of butylone (demethylenation metabolite) was identified by MRM transitions at m/z 336>294 and 336>174 (butylone-M dihydroxy-3Ac). Nor-butylone was identified by MRM transitions at m/z 250>160 and 250>132 (bk-BDB-Ac). Finally, the 4-OH-3-MeO metabolite was identified by MRM transitions at m/z 308>248 and 308>174 (butylone-M (demethylenyl-methyl-) 2Ac). Derivatives of MDMA and its metabolites were stable in the HPLC mobile phase for 30 h at room temperature and were quantified in all 10 positive human urine samples. HMMA was the major metabolite in human urine samples whereas HHMA was the major metabolite by HLM. However, HHMA is an intermediate metabolite leading to HMMA. This result confirms that demethylenation of the methylenedioxy ring, followed by catechol-O-methyltransferase (COMT)-catalyzed methylation is a major metabolic pathway of MDMA in humans.

Conclusion: Human liver microsomes can be used to simulate drug metabolism in humans and to provide chromatographic and mass spectrometric data on metabolites. Acetate derivatives result in higher molecular weights, providing more specific ions for identification, and in decreased polarities of metabolites, improving their analysis on reversed phase C18 columns. This method would be suitable for routine analysis of urine to detect and confirm abuse of methylenedioxy-substituted amphetamines.

Keywords: Methylenedioxy-Substituted Amphetamines, *In vitro* Metabolism, Human Liver Microsomes

P70

Development of a Latex-Enhanced Immunoturbidimetric Assay for the Detection of Ethyl Glucuronide in Urine Samples

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Introduction: Ethyl glucuronide (EtG) is a phase II metabolite of ethanol which has found application as a biomarker for the detection of recent alcohol consumption. EtG is formed by the conjugation of ethanol to glucuronic acid and accounts for 0.5% of total alcohol elimination. It can be detected in urine for up to 4 days following exposure to alcohol, giving it a much longer detection window than that of unmodified ethanol. The detection of alcohol consumption has a number of important applications, such as diagnosis and treatment of alcohol intoxication or poisoning, monitoring of individuals in professions with zero-tolerance policies with respect to alcohol, determination of legal impairment and forensic judgement. Latex-enhanced immunoturbidimetric assays due to short reaction times and their application to automated analysers, are valuable analytical tools for the high-throughput screening of samples.

Objective: The aim of this study was to develop a latex-enhanced immunoturbidimetric assay for the detection of EtG in urine and applicable to a variety of automated clinical chemistry analysers.

Method: In this latex-enhanced immunoturbidimetric assay, the latex particles are coated with EtG which binds to the specific antibody in the solution causing agglutination. When a sample containing EtG is added, the agglutination is inhibited to an extent proportional to the amount of EtG in the sample. The change in turbidity caused by agglutination is measured as a change in absorbance, which is inversely proportional to the concentration of EtG in the sample. The assay is qualitative utilising a 500 ng/mL cut-off and is applicable to a variety of analysers. The results reported were obtained with an Rx daytona analyser.

Result: The assay was standardised to EtG and methyl ethyl glucuronide was also detected with a cross-reactivity (%) value of 61.5. The Limit of Detection in urine (n=20) (determined as the mean concentration + 3 standard deviations) was 213 ng/mL (measuring range: 0-2000 ng/mL). Human negative urine was spiked with the following levels of EtG: 375 ng/mL (negative sample), 500 ng/mL and 625 ng/mL (positive sample). The intra-assay precision, determined by assessing 20 replicates of each urine sample, was calculated as 3.35% at 375 ng/mL, 2.81% at 500 ng/mL and 1.82% at 625 g/mL. Recovery was assessed with a set of authentic human urine samples (n=32) and 94% were correctly reported as negative and positive with respect to values determined by LC-MS.

Conclusion: The results show optimal performance of this latex-enhanced immunoturbidimetric assay for the determination of EtG in urine samples. The assay is applicable to different automated analysers and utilises ready–to-use reagents, which ensures the reliability and accuracy of the measurements and facilitates the testing procedure. The assay is of value as an analytical tool in medical and legal applications.

Keywords: Ethyl Glucuronide, Latex-Enhanced Immunoturbidimetric Assay, Ethanol

P71

Development of a New Latex-Enhanced Immunoturbidimetric Assay for the Detection of UR-144/XLR-11 and their Metabolites in Urine Applicable to a Variety of Automated Analysers

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Introduction: Synthetic Cannabinoids are chemical compounds that mimic the effects of tetrahydrocannabinol, the main active ingredient of cannabis. The generation of new synthetic cannabinoids is continuously evolving, to circumvent legal restrictions. UR-144 [(1-pentyl-1H-indol-3-yl)-(2,2,3,3-tetramethylcyclopropyl) methanone] and its fluorinated version XLR-11 (5-fluoro UR-144) are the new generation of synthetic cannabinoids. UR-144 is a synthetic derivative of the first generation synthetic cannabinoid JWH018 [naphthalene-1-yl(1-pentyl-1H-indol-3-yl)methanone] in which the naphthalene ring is substituted with a tetramethylcyclopropyl group. UR-144 and XLR-11 are potent and addictive and as these designer drugs continue to be sold there is a need for screening tests in the detection process. UR-144 N-pentanoic acid is one of the primary urinary metabolite of UR-144 and can be utilized to screen the abuse of UR-144. The use of immunoturbidimetric assays applicable to a variety of automated clinical chemistry analysers facilitates the screening process in test settings by increasing the screening capacity.

Objective: This study reports the development of a new latex-enhanced immunoturbidimetric assay targeted to the metabolite UR-144 N-pentanoic acid in urine. The assay is applicable to a variety of automated clinical chemistry analysers.

Method: The assay is based on a latex-enhanced immunoturbidimetric assay. The analyte is coated on the latex particles and binds to the specific antibody in the solution, which causes agglutination. When a sample containing the analyte is added the agglutination is inhibited to an extent proportional to the amount of the analyte in the sample. The change in turbidity caused by agglutination is measured as a change in absorbance, which is inversely proportional to the concentration of the analyte in the sample. The assay is qualitative utilising a 10 ng/mL cut-off and is applicable to a variety of automated analysers. In this study the results presented were obtained with a RX daytona analyser using neat urine samples.

Result: In this initial evaluation the assay was standardised to the metabolite UR-144 N-pentanoic acid, the parent compound UR-144 presented a cross-reactivity (%) value of 25. Other metabolites and synthetic cannabinoids were also detected with cross-reactivity (%) values ranging from 28 [UR-144 N-(5-chloropentyl) analog] to 94 (A-796260). XLR-11 metabolites were also detected with cross-reactivity (%) values ranging from 33 [XLR11 N-(4-pentenyl) analog] to 49 [XLR11 N-(3-fluoropentyl) isomer].The limit of detection in urine (n=20) (determined as the mean concentration + 3 standard deviations) was 2.8 ng/mL (assay range: 0-20 ng/mL). For the determination of the precision and the recovery, human negative urine was spiked with three different concentrations of the UR144 N-pentanoic acid at 5 ng/mL, 10 ng/mL and 15 ng/mL. The intra-assay precision (n=20), was calculated as 5.81% (5 ng/mL), 6.45% (10 ng/mL) and 5.94% (15 ng/mL). The mean recovery for all the samples (n=20) at the three concentrations was between 98-105%. All replicates were correctly reported as negative and positive.

Conclusion: The results of this initial evaluation show applicability of the assay for the determination of UR-144/XLR-11 and their metabolites as well as other synthetic cannabinoids in urine samples. The assay is applicable to different automated analysers and utilises ready–to-use reagents which ensures the reliability and accuracy of the measurements and facilitates the testing procedure. The assay is of value as a new analytical tool to screen for these new generation designer drugs in clinical laboratories.

Keywords: UR-144, Latex-Enhanced Immunoturbidimetric Assay, XLR-11

P72

A Fast and Comprehensive Analysis of 32 Synthetic Cannabinoids Using Agilent Triple Quadrupole LC/MS

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Introduction: Synthetic cannabinoids are a group of psychoactive compounds that mimic the effects of Δ9-tetrahydrocannabinol, the primary psychoactive constituent of marijuana (*Cannabis sativa L)*. The Drug Enforcement Administration (DEA) has classified many of the most common cannabinoids, including JWH-018, JWH-073 and their derivatives, as Schedule 1 controlled substances. Chemists, seeking to avoid prosecution under DEA scheduling regulations, are continually synthesizing new cannabinoids. As a result, several novel synthetic cannabinoids have emerged in the illicit drug market including: PINACA, FUBINACA, PB-22, AKB-48 and multiple derivatives of these compounds.

Objective: Our laboratory sought to develop and validate an analytical method for the analysis of a broad range of synthetic cannabinoid metabolites in urine samples.

Method: The proposed method has been validated following the Food and Drug Administration Bioanalytical Method Validation guidelines. 32 synthetic cannabinoids were evaluated: JWH-073 N-propanoic acid, JWH-073 N-butanoic acid, JWH-073 N-(4-hydroxybutyl), JWH-200 5-hydroxyindole, JWH-203 N-pentanoic acid, JWH-210 N-(5-hydroxypentyl), JWH-018 N-pentanoic acid, JWH-018 N-(5-hydroxypentyl), JWH-019 N-(6-hydroxyhexyl), JWH-081 N-(5-hydroxypentyl), JWH-122 N-(5-hydroxypentyl), JWH-250 N-(5-hydroxypentyl), JWH-398 N-pentanoic acid, AKB-48 N-pentanoic acid, AM2201 N-(4-hydroxypentyl), AM-694 N-pentanoic acid, MAM2201 N-pentanoic acid, 5-fluoro-PB-22 carboxyindole, PB-22 3-carboxyindole, BB-22 3-carboxyindole, RCS-4 N-(5-hydroxypentyl) phenol, RCS-4 N-(5-hydroxypentyl), UR-144 N-pentanoic acid, UR-144 pyrolysis N-pentanoic acid, XLR-11 6-hydroxyindole, XLR-11 N-(4-hydroxypentyl), AB-PINACA N-(4-hydroxypentyl), AB-FUBINACA metabolite, AB-PINACA pentanoic acid, ADB-PINACA pentanoic acid, ADBICA N-pentanoic acid, and 5-fluoro-AB-PINACA N-(4-hydroxypentyl). Following enzymatic hydrolysis using β-glucuronidase, target analytes were recovered by liquid-liquid extraction utilizing 1-chlorobutane: Isopropyl alcohol (70:30) as the organic ratio. Chromatographic separation was achieved using an Agilent Technologies 1290 liquid chromatograph equipped with Zorbax Eclipse Plus C18 columns (2.1mmx50mm 1.8um). Mobile phases consisted of 0.2% acetic acid in DI water (A) and 100% acetonitrile (B). Positive identification was made using and Agilent Technologies 6460 triple quadrupole mass spectrometer (QQQ) with a Jetstream electrospray source operating in positive ion mode with the following common parameters: nitrogen drying gas temperature 350°C, nitrogen sheath gas temperature 400°C, nitrogen drying gas flow 10 L/min, nitrogen sheath gas flow 11 L/min, nebulizer pressure 55 psi, capillary voltage 5000 V, and nozzle voltage 1000 V. The dynamic MRM method monitored for 81 transitions. One MRM transition served as a quantifier transition and a second MRM transition served as a qualifier transition for all drugs. One MRM transition was used to monitor internal standards. All qualifier ion ratios were determined to be within +/- 20% of calibrator qualifier ion ratios. The chromatographic run time was 4.00 minutes with a concentration range of 0.5-200 ng/mL.

Result: Linearity, accuracy and precision of the proposed method were within the acceptable criteria. Interday (N=15) accuracy of quality control samples ranged from 83-115%, while precision (RSD, N=15) ranged from 5-11%. The recovery of target analytes from spiked human urine ranged from 10% to 85%. Matrix effect ranged from 3-60%. Selectivity, specificity, interference and carryover studies were also investigated. This method has shown to be selective and specific, providing no evidence of interference or carryover concerns. Finally, 25 donor samples from suspected synthetic cannabinoid users were analyzed. 10 distinct synthetic cannabinoids were detected, representing a positivity rate of 88% of all tested samples. This method has been validated for urine matrix only; however several research publications exist where the detection of synthetic cannabinoids was investigated in oral fluid and serum matrices as well.

Conclusion: Detection and quantitation of newly synthesized synthetic cannabinoids has been a challenge in a scientific community. Laboratories providing synthetic cannabinoid analyses should continuously look to expanding their testing panels. The data presented here represents a validated LC/MSMS method to accurately identify and quantitate 32 synthetic cannabinoids metabolites in urine samples. Included in this method are metabolites that are constituents of the new generation of synthetic cannabinoids.

Keywords: Synthetic Cannabinoids, LC/MSMS, Toxicology, Method Validation

P73
Stability of Drug Standards Over Time: A Study of UV-vis Absorbance

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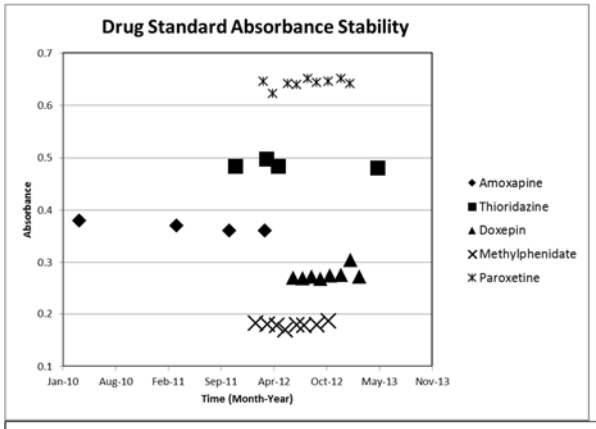
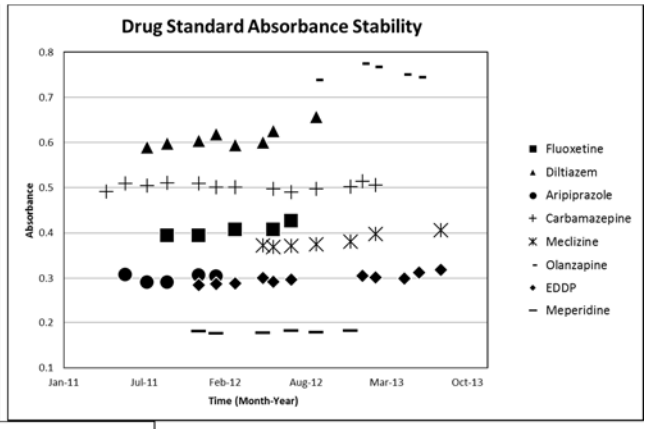
Introduction: The quality of toxicological analysis is directly impacted by the accuracy of the concentration measurements that are based on comparison to reliable and consistent drug reference materials. Drug standard solutions used to make calibrators and controls may be utilized up to a year without regular evaluation.

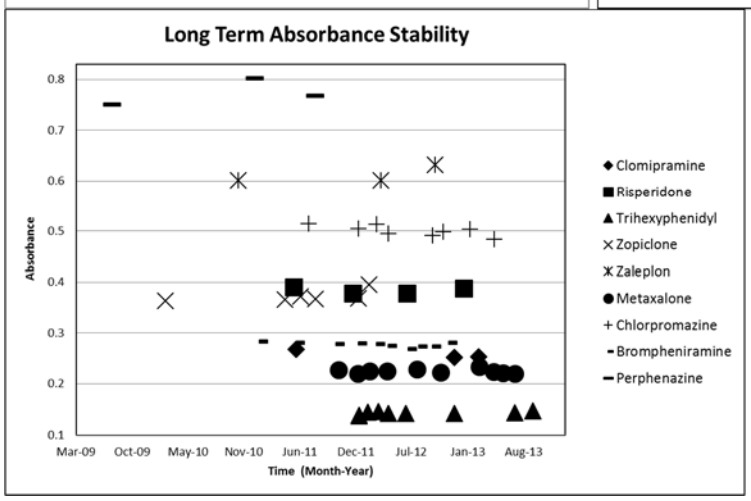
Objective: To evaluate the stability of drug standard stock solutions by monitoring the UV-vis absorbance over time.

Method: A variety of prescription antidepressants, antipsychotics and narcotic analgesics of pharmaceutical purity, obtained from various vendors including Sigma-Aldrich and U.S. Pharmacopeial (USP) Convention, were tested. Examples include doxepin, thioridazine, and meperidine. Total collection intervals varied approximately from 6 months up to 2 years with data points collected as frequently as every month. Method, frequency and length of data collection based on type of drug. The stability of drugs in organic solvents stored in the freezer (-20°C) was monitored over time via the measurement of UV-visible (UV-vis) absorbance on an Agilent 8453 UV-vis spectrophotometer, where the initial data point comes from a freshly made solution. Standards were allowed to stand at room temperature for up to an hour before obtaining data. Dilutions were made in methanol, 0.5 N sulfuric acid, or ethanol.

Result: Absorbance values were compared to a 10% range established by the literature or historical data. The standard deviation ranged from 0.002-0.03.







Conclusion: UV-vis absorbance of drugs standards exhibited little variability for short as well as long intervals, validating the practice of using various drug stock standards for up to two years. The stability of drugs tested frequently did not suffer after repeatedly warming solutions to room temperature.

Keywords: Standard, UV-vis, Stability

P74

Improved Chiral Separation of Methamphetamine Enantiomers Using CSP LC-MS/MS

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Introduction: To determine the true enantiomeric composition of methamphetamine urine drug testing results, further chiral separation of the d and l enantiomers is necessary. For example, positive detection of l-methamphetamine can be attributed to multiple possibilities including but not limited to the legal OTC Vick's Inhaler, while d-methamphetamine is usually attributed to legal prescription drugs to treat ADHD or obesity, or the illegal recreational form (e.g, crystal meth). While enantiomeric separation of methamphetamine has traditionally been accomplished using GC-MS, chiral separation of dextro (d) and levo (l) methamphetamine by Chiral Stationary Phase (CSP) LC-MS/MS has proven more difficult.

Chirally selective detection of methamphetamine by GC/MS is often performed using a derivatizing agent, L-N-trifluoroacetyl-prolylcholoride (TPC). L-TPC, a chiral compound, is known to have impurities which can affect the chiral composition percentages of the methamphetamine sample. Previous studies demonstrated racemization by L-TPC result upwards of 12% conversion. This suggests the possibility of inaccurate patient results. This study illustrates that with the use of a specific type of CSP LC in tandem with mass spectrometric detection to perform chiral separation and detection of methamphetamine in human urine yields more accurate determination of the enantiomeric composition of the sample and therefore, the source of that sample.

Objective: To develop a CSP-LC-MS/MS method for analyzing the ratio of d to l methamphetamine.

Method: Standards and samples were run on either GC-MS or CSP-LC-MS/MS. GC-MS analysis was performed by first derivatizing with L-TPC and then extracting with GV-65 cartridges with analysis on a GC Agilent 5975 MSD with a 15m DB-5 0.25µ column at a run time of 6.3 minutes. CSP LC samples were diluted with phosphate buffer and extracted using Cerex Trace B cartridges. Eluates were dried down and reconstituted in methanol. Injections were separated with an Astec Chirobiotic™ V2 15cm x 4.6mm, 5 µm column on a Waters Acquity TQD. The total run time for the method was 8 minutes using an isocratic solvent flow of 95:5:0.1:0.2 methanol:water:acetic acid:ammonium hydroxide and was linear from 50 ng/mL to 2500 ng/mL. This assay monitors two transitions for S(+)-methamphetamine (d), R(-)-methamphetamine (l) and the internal standard, methamphetamine-D5.

Result: Comparative analysis of samples run by GC and LC methods showed preferential bias of the GC method for producing results that were never 100% d or l isomer. This favors a theory that either a chiral impurity of L-TPC or the racemization of methamphetamine during derivatization produces an error, consistent with previous research, of 8-19% (of total area) at various concentrations. Our studies show that the CSP-LC-MS/MS method produces percent deviation errors of less than 2% when using pure d and l standards. Unknown patient samples ran on the CSP method also demonstrate reduced percent conversation rates compared to the GC-MS method. Per HHS guidelines, a sample containing more than 20% d-methamphetamine is considered to be from a source other than the OTC "l" form.

Conclusion: A higher rate of d- and l-methamphetamine isomer racemization is seen in samples when analyzed by GC-MS using L-TPC derivatizing agent. This racemization is not seen when these samples are tested with CSP-LC-MS/MS. Thus, a more accurate method of enantiomeric analysis is provided by CSP-LC-MS/MS.

Keywords: Methamphetamine, Isomer, Testing

P75
Detection of Drugs of Abuse in Nails: Three Year Experience

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Background: Nails (fingernails and toenails) and hair are made of keratin. Substances incorporate into the keratin fibers. Nails are thicker than the typical strand of hair and are capable of capturing more of a substance than hair. Drug use can be revealed by nail analysis 1-2 weeks later. The time period during which drug or alcohol ingestion can be detected for 3-5 months in fingernails, and 8-14 months in toenails. Data of drug/metabolite concentrations in nails are limited.

Objective: We survey the drug testing results from 10,349 nail samples submitted to our laboratory from high risk cases during a 3-year period of time between 2012 and 2014.

Method: Nail samples were collected by clipping of 2-3 mm long from all nails (approximately 100 mg). All samples were analyzed by validated analytical procedures. The initial testing was performed using ELISA with the exception of ethyl glucuronide (EtG), buprenorphine, ketamine, naltrexone, naloxone, butorphanol, and propofol glucuronide for which the instrumental screening by LC-MS-MS was employed. Presumptive positive samples were then subjected to confirmatory testing, with sample preparation procedures including washing, pulverizing, digestion, and extraction optimized for each drug class. LC-MS-MS was used for confirmatory analysis of majority of drug classes, including the above drugs mentioned. Exceptions were phencyclidine, barbiturates, tramadol, and normeperidine being confirmed by GC-MS, and carboxy-THC was confirmed by GC-GC-MS-MS.

Result/Discussion: The total of 7,799 samples were analyzed for amphetamines (amphetamine, methamphetamine, MDMA, MDA, MDEA). The concentrations ranged from 40-572,865 pg/mg (median 100-3,687) for all amphetamine analytes. Amphetamine and methamphetamine were present in approximately 14% of the nail samples, only 22 samples were found positive for MDMA (0.3%), 7 for MDA (0.09%), and 4 for MDEA (0.05%). Cocaine and related analytes (benzoylecgonine, norcocaine, cocaethylene) were found in 5% samples (7,787 total), and a concentration range was 20-265,063 pg/mg (median 84-1,768). Opioids overall ranged from 40 to 118,229 pg/mg (median 123-830). The most prevalent opioid was oxycodone (15.1%) and hydrocodone (11.4%), compared to 1.5-3.6% for the others including morphine, codeine, hydromorphone, methadone, EDDP, and oxymorphone. Carboxy-THC positivity rate was 18.1% (0.04 to 262 pg/mg, median 6.41). Out of 3,039 samples submitted for EtG analysis, 756 were positive (24.9%), and a concentration range was 20-3,754 pg/mg (median 88). Examples of other drugs found in fingernails included barbiturates, benzodiazepines, ketamine, meperidine, tramadol, zolpidem, propoxyphene, naltrexone, and buprenorphine. For the substances we tested for both parent drug and correspondent metabolite, parent drugs were generally higher concentrations than their metabolites. Naltrexone was an exceptional case where its metabolite 6-β-naltrexol, not a parent drug, is the predominant form present in nails. In addition, much smaller sample sizes and tested analytes were available to compare drug concentrations between the fingernails and toenails. Mean concentrations of drugs/metabolites in fingernails were higher than in toenails, except for amphetamine mean concentration was 1,879 pg/mg in fingernails (n = 212), and 2,906 pg/mg in toenails (n = 99).

Conclusion: During the last several years nail analysis has become a reliable way of determining the long-term use and abuse of drugs and illicit substances. Improved extraction techniques are simple and produce accurate and precise results. Very sensitive analytical instrumentation, mainly LC-MS-MS, allows for detection of very low concentrations of substances and biomarkers in nails. The samples were from a high-risk population, therefore the extraordinary positivity rate was observed.

Keywords: Nail Analysis, Keratinized Specimens, Drugs of Abuse

P76

Field Evaluation of a Proof-of-Concept Homogeneous Enzyme Immunoassay for the Detection of Buprenorphine, Norbuprenorphine and their Glucuronide Metabolites

Paul V. White*; Preferred Laboratory, Worcester, MA

Background/Introduction: Thermo Fisher is in the process of developing a new Buprenorphine Assay (Enzyme Immunoassay) that can measure Buprenorphine and Norbuprenorphine along with the glucuronide conjugates of both Buprenorphine and Norbuprenorphine in human urine. The assay is intended for the qualitative and semi-quantitative detection of Buprenorphine and its metabolites in human urine at a cutoff of 10 ng/mL on clinical chemistry analyzers. Currently available commercially immunoassays either detect only Buprenorphine and Buprenorphine Glucuronide or detect only Buprenorphine and Norbuprenorphine while this new Buprenorphine Assay detects all.

Objective: The objective of this study was to perform an initial field evaluation of the proof-of-concept homogenous enzyme immunoassay that can detect Buprenorphine, Norbuprenorphine, Buprenorphine Glucuronide and Norbuprenorphine Glucuronide and compare Liquid Chromatography Tandem Mass Spectroscopy (LC-MS/MS) and the current Thermo Scientific CEDIA® Buprenorphine Immunoassay

Method: Studies were performed in semi-quantitative mode on a Beckman Coulter AU400® clinical chemistry analyzer. Thermo Fisher Scientific provided assay parameters, reagents, calibrators and controls for this study. Using a 10 ng/mL cut-off with 7.5 and 12.5 ng/mL controls intra-run precision was performed using 20 replicates of both control levels. Linearity was assessed by 2-fold serial dilution of the high calibrator (50 ng/mL) and determining the recovery. Method comparison was performed by testing 192 residual urine samples using both EIA methods and LC-MS/MS. Quantification of all free and total buprenorphine and norbuprenorphine was by LCMS/MS.

Result: Intra-run precision of the controls showed acceptable precision. The Low control showed a CV of 5.7% while the High control showed a CV of 3.3%. Serial dilution study demonstrated good recovery for 50, 25 and 12.5 ng/mL samples (105 – 118% recovery) with an r value of 0.998. Method comparison study showed 99.5% agreement between the new CEDIA Buprenorphine Assay, CEDIA Buprenorphine/Norbuprenorphine, and LC-MS/MS. The study also showed a 99.5% agreement between the original CEDIA Buprenorphine Assay and the new concept Buprenorphine assay.

Total Samples -192
Positive agreement CEDIA Buprenorphine to CEDIA Buprenorphine/Norbuprenorphine to LCMSMS 121 samples
Negative agreement CEDIA Buprenorphine to CEDIA Buprenorphine/Norbuprenorphine to LCMSMS70 samples
Negative for CEDIA Buprenorphine to Positive CEDIA Buprenorphine/Norbuprenorphine to Negative for LCMSMS 1 sample

Conclusion: The concept Buprenorphine Assay demonstrated good precision and linearity. Improvement in specificity and sensitivity was noted in comparison to LCMS/MS. Further studies are needed to examine calibration stability, controls recovery as well as testing more patient samples.

NOTE: *The assay is currently in development and is not approved by FDA*

Keywords: Buprenorphine, Immunoassay, Norbuprenorphine

P77

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 and Paul Connolly; 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: Multi-Class Drugs, Screening Method, LC-MS/MS, Raptor™ Biphenyl, Therapeutic Drug Monitoring

P78

A Rapid and Accurate LC-MS/MS Method for the Analysis of Nicotine, Nicotine Metabolites, and Minor Tobacco Alkaloid in Urine

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Background/Introduction: The analysis of nicotine metabolites has several aspects including monitoring public tobacco exposure, evaluation of nicotine replacement therapy, drug therapy assessment, forensic toxicology analysis, and life or health insurance applications. Most of the modern methods adapt the usage of high pH mobile phases with relatively high concentration of additive reagents, which may not be applicable to all LC-MS instrumentation. An LC-MS/MS method was developed for urine testing of nicotine, cotinine, trans-3'-hydroxycotinine, nornicotine, norcotinine, and anabasine. It was demonstrated that a fast and highly efficient analysis of these basic compounds can be achieved with the Raptor™ Biphenyl column using regular solutions suitable for all LC-MS/MS instrumentation.

Objective: The intent of this study was to develop a method for the analysis of nicotine related compounds in urine using "friendly" LC-MS/MS mobile phases and the highly efficient and selective Raptor™ Biphenyl column.

Method: Fortified standards and QC samples were prepared with a liquid-liquid extraction procedure. An aliquot of 250 µL urine was mixed with 50 µL of internal standard solution (250 ng/mL of each deuterated analyte in methanol) and 50 µL of 5N NaOH in a 4 mL glass vial. Extraction was performed by adding 1.5 mL of 50/50 methylene chloride/diethyl ether and stirred for 1.5 minutes. After centrifugation at 4000 rpm for 5 minutes, 1 mL of organic phase was transfer to a 1.5 mL HPLC vial and mixed with 10 µL of 0.25N HCl before evaporating to dryness at 35°C under a gentle stream of nitrogen. The dried extract was reconstituted with 200 µL of water and injected on to the Raptor™ Biphenyl column (100x2.1mm, 5µm) for the analysis with the Waters ACQUITY UPLC® I-Class System coupled with a Waters Xevo® TQ-S mass spectrometer using electrospray ionization in positive ion mode. The mobile phases used are 0.1% formic acid, 5mM ammonium formate in water and 0.1% formic acid in methanol.

Result: The linearity range was from 2-1000 ng/mL for nicotine, nornicotine, norcotinine, and anabasine; 5-5000 ng/mL for cotinine; 10-5000 ng/mL for trans-3'-hydroxycotinine. The r^2 was ≥ 0.995 and the % deviation was within 15% of the nominal concentration (≤ 20% for the lowest concentrated standard). Per the noise-to-signal value at 10, the LLOQ is 0.4 ng/mL for all analytes. Three levels of QC samples (7.5, 75, and 750ng/mL for nicotine, nornicotine, norcotinine, and anabasine; 75, 750, and 10,000 ng/mL for cotinine and trans-3'-hydroxycotinine) were prepared in urine. The 10,000 ng/mL QC sample for cotinine and trans-3'-hydroxycotinine was diluted 5-fold in water for the analysis. Analyses were performed on 3 different days. The method accuracy was demonstrated from the %recovery of within 10% of the nominal concentration for all QC levels. The %RSD was from 0.6-8.2% and 1.4-9.9% for intra-day and inter-day, respectively. With the established method, anabasine could only be detected in tobacco users' urine.

Conclusion/Discussion: The Raptor™ Biphenyl column was demonstrated to be excellent for simultaneous analysis of nicotine, two major metabolites (cotinine and trans-3'-hydroxycotinine), two minor metabolites (nornicotine and norcotinine), and a minor tobacco alkaloid, anabasine, in human urine. The accurate and reproducible analysis can be achieved in less than 5 minutes of chromatographic run time and is thus applicable to low-cost and high through-put analysis of nicotine related compounds.

Keywords: Nicotine, Metabolites, Anabasine

P79

The Isolation and Distribution of Synthetic Cannabinoids in Two Postmortem Cases

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Introduction: Synthetic cannabinoids have exploded on the recreational drug market in recent years due to their ability to produce psychoactive cannabis-like effects. These compounds have no approved medicinal purpose though numerous emergency room visits have been reported in conjunction with their use. Some of the symptoms reported include: excited delirium, seizure, coma, agitation, myocardial infarction, convulsions, difficulty breathing, and an altered state of consciousness. While there is limited safety or toxicological information available in the scientific literature regarding synthetic cannabinoids, there have been several deaths attributed nationally to their use. The AFMES Division of Forensic Toxicology laboratory has encountered 2 deaths in which synthetic cannabinoids were involved - one death was attributed to AB-CHMINACA use and the other had XLR11 and UR144 metabolites present. Biological specimens from both autopsies were subsequently submitted for toxicological analysis.

Objective: To present parent and metabolite synthetic cannabinoid quantitative values and their distribution into various matrices.

Method: Separate aliquots of each specimen submitted for analysis were evaluated for both parent and the metabolic byproducts of synthetic cannabinoid ingestion. Parent synthetic cannabinoids were isolated by alkaline liquid-liquid extraction. Specimens used for synthetic cannabinoid metabolite extraction were first subjected to an enzymatic hydrolysis followed by an acidic liquid-liquid extraction. Both sets of samples were analyzed using liquid chromatography tandem mass spectrometry operating in the multiple reaction monitoring mode (MRM).

Result: Parent and metabolite synthetic cannabinoid concentrations for each case are displayed in the table below.

Table 1. Drugs Present in Various Matrices in ng/mL or ng/g

		Parent Compounds			Metabolites			
		AB-CHMINACA	AKB48	XLR11	AB-CHMINACA Metabolite 4	UR144-N-COOH	UR144-N-OH	XLR11-N-OH
Case 1	Central Blood			1.95		48.40	8.94	
	Peripheral Blood			2.10		45.20	6.11	
	Urine			< LOQ		94.60	77.63	
	Vitreous			< LOQ		0.82	< LOQ	
	Liver			0.31		> LOL	23.70	Present
	Heart			6.47		27.40	9.72	
	Kidney			4.64		51.60	26.21	
	Spleen			7.96		19.20	9.28	
	Lung			1.29		36.80	21.53	
	Brain			5.34		1.99	4.34	
	Adipose			490.47		10.80	25.06	
	Gastric			882.79		64.00	39.00	
Case 2	Peripheral Blood	10.21	0.36	0.09	1.63	0.54	0.24	
	Urine	0.79			3.41	1.67	0.72	
	Vitreous	< LOQ						
	Liver	47.82	1.03	< LOQ	12.80	7.60		
	Heart	24.22	2.11	0.33	< LOQ	1.47		
	Spleen	26.16	0.55	0.15	1.04			

Case 1: A 24 year old male failed to show up for duty and was found unresponsive in a locked room. No signs of foul play were evident. An initial investigation of the scene revealed a can of aerosol dust remover ("Dust-off"), an empty "spice" packet, prescribed medication, a prescription bottle containing unknown green leafy material, and a homemade smoking device. Vascular congestion of the liver and spleen were noted during autopsy. Furthermore, both lungs displayed diffuse congestion and edema, with the right lung weighing 840 g and the left lung 780 g. In addition to XLR11, 1,1-difluorethane was qualitatively identified in the blood and vitreous fluid by gas chromatography/mass spectrometry (GC/MS). Toxic levels of hydrocodone were also confirmed in the blood (0.16 mg/L) and urine (3.94 mg/L) using GC/MS.

Case 2: A 24 year old male was found deceased on his front porch with a lighter in his hand and a pack of "spice" on the ground near the body. The autopsy revealed no evidence of acute injury or natural disease, though emesis and dried blood were observed around the nostrils and mouth.

Conclusion: AB-CHMINACA, XLR11, and their respective metabolites were quantitated in a variety of matrices. AKB48 was also quantitated but no metabolites were detected. XLR11 is the fluorinated version of UR144. The presence of the parent cannabinoid XLR11 along with the XLR11-N-OH, UR144-N-COOH and UR144-N-OH metabolites demonstrate the defluorination metabolic route for compounds containing a terminal fluorine on the N-alkyl side chain. MRM transitions for UR144 were monitored but not detected in any of the matrices. The two postmortem cases provide quantitative toxicological information for the forensic community to assist in interpreting toxicology casework. The analysis also allows for correlation between parent synthetic cannabinoids and their corresponding metabolites in a variety of matrices.

Keywords: Synthetic Cannabinoids, Postmortem, Distribution

P80

Toxicological Screen of Postmortem and Antemortem Samples for Basic, Acidic, and Neutral Drugs Utilizing Liquid Chromatography/Time-of-Flight Mass Spectrometry

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Background/Introduction: Forensic toxicology laboratories often work to improve screening methods by decreasing sample volume and analysis time, while increasing both sensitivity and selectivity. Time-of-flight mass spectrometry (UPLC-MS^E-QTOF) has allowed for the latter by utilizing MS^E mode to collect exact mass precursor ion and fragment ion spectra. The majority of published methods developed for this instrumentation have focused solely on basic and neutral drugs. This study focused on the development and validation of a method that encompassed basic, neutral, and acidic drugs in multiple matrices. The results were compared to validated in-house methods for acid/neutral and basic drug screens as analyzed by GC-MS/NPD.

Objective: The goal of this study was to develop a comprehensive toxicological screen utilizing a single solid-phase extraction to analyze for basic, acidic, and neutral drugs, thus combining two in-house screening methods. It was necessary for the resultant method to be selective and have increased sensitivity, along with decreased sample volume.

Method: A reference standard containing sixty-eight drugs was spiked into nine different sample matrices to conduct the method validation. Three milliliters of 0.1M pH 6 sodium phosphate buffer was added to a 1 mL sample volume. The pretreated samples were extracted using UCT Clean Screen® columns. The columns were conditioned, washed and dried. The acidic drugs were eluted with a hexanes/ethyl acetate solution. The columns were washed and dried again. The basic/neutral fraction was collected separately, after being eluted with an isopropanol, ammonium hydroxide, and methylene chloride solution. Both elution fractions were dried at 40 °C under nitrogen and reconstituted in their respective initial mobile phases. The basic/neutral fraction was analyzed using the Waters Xevo G2 QTOF in positive electrospray ionization mode on a Waters Acuity UPLC HSS C18 column (2.1 x 150 mm) and eluted with a gradient of 5mM pH 3 ammonium formate and 0.1% formic acid in acetonitrile. The acidic fraction was analyzed in negative electrospray ionization mode on a Waters Acuity UPLC BEH C18 column (2.1 x 50mm) and eluted with a gradient of 5mM pH 8.5 ammonium acetate and acetonitrile.

Result: The method was validated on the following considerations: selectivity, limit of detection, intra/inter-assay precision, matrix effects/ion suppression/enhancement, and carryover. There were no interferences with the 68 drugs in this study. Forty-two basic/neutral drugs were detected at 5 ng/mL. The remaining were detected at 10 ng/mL and 25 ng/mL. Acidic drugs were identified from 100 ng/mL to 5000 ng/mL. All basic drugs had a coefficient of variation (CV) less than 15%. Of the acid/neutral drugs 66% had a CV less than 15%. Fifty drugs were suppressed or enhanced greater than ± 25% in one of nine matrices and no carryover was detected. This method proved to be more sensitive than the GC-MS/NPD methods, while requiring half the sample volume, extraction and instrumental analysis time, and data processing time.

Conclusion/Discussion: This toxicological screen proved to be selective, sensitive, and reproducible through validation with some variance due to matrix effects. Further work is necessary to enhance capabilities for complex matrices. Developing a single extraction method that combined two in-house methods optimized laboratory efficiency by increasing sensitivity and decreasing both sample volume and overall analysis time.

Keywords: Toxicological Screen, Time of Flight Mass Spectrometry, Solid-Phase Extraction

P81

Identification of Fifteen Metabolites of the Designer Hallucinogen 25I-NBOMe in Human Urine

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Introduction: The identification and analysis of metabolites of new compounds appearing on the designer psychoactive drug market is crucial, since these metabolites can serve as biomarkers for drug exposure. Recently, a new class of designer hallucinogenic drugs, dimethoxyphenyl-N-[(2-methoxyphenyl) methyl]ethanamine) (NBOMe) derivatives, have become widely available via the Internet. Currently, 2-(4-iodo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl) methyl]ethanamine (25I-NBOMe) is the most popular abused derivative. There is little data concerning absorption, metabolism and elimination of 25I-NBOMe in man, and therefore, there are no definitive metabolite biomarkers.

Objective: To identify potential human urinary biomarker metabolites of the designer hallucinogen 25I-NBOMe in cases of ingestion or exposure.

Method: 25I-NBOMe was incubated with freshly prepared mouse liver microsomes. These *in vitro* specimens were analyzed for phase I and phase II metabolites using both a Waters Acquity Xevo TQD LC-MS/MS system and a Shimadzu multidimensional GC-MS QP-2010 Ultra with EI ionization (Shimadzu Scientific Inc., Columbia, MD) equipped with a Dean's switch. This study was approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The metabolites identified in this study were then screened for in two human urines from suspected 25I-NBOMe users

Result: A total of fifteen phase I and phase II metabolites of 25I-NBOMe were identified in the mouse liver microsomes. The identified phase I metabolites were 2-((2-(4-iodo-2,5-dimethoxyphenyl) ethylamino) methyl) phenol (25I-NBOH); 2-iodo-4-methoxy-5-[2-[(2-methoxyphenyl)methylamino]ethyl]phenol (2-O-desmethyl-5-I-NBOMe); 5-iodo-4-methoxy-2-[2-[(2-methoxyphenyl)methylamino]ethyl]phenol (5-O-desmethyl-2-I-NBOMe); 2,5-di methoxy-4-iodo phenethylamine (2C-I); 5-(2-aminoethyl)-2-iodo-4-methoxy-phenol (5-OH-2C-I);2-(2-aminoethyl)-5-iodo-4-methoxy-phenol (2-OH-2C-I), an aromatic ring hydroxylated metabolite designated 25I-NBOMe-OH, and an unidentified O-desmethyl metabolite. All of these metabolites, except 2C-I, were also detected as phase II glucuronide metabolites.

The fifteen identified metabolites and the parent drug, 25I-NBOMe, were detected in one of the human urine specimens. The non-glucuronidated metabolites were only detected in trace amounts. After ß-glucuronidase enzymatic hydrolysis of this urine specimen, no glucuronide metabolites were detected, while the previously non-glucuronidated metabolites were detected in greater abundance. The O-desmethyl metabolites, as well as 2C-I, were also detected in greater abundance than the parent 25I-NBOMe. In the second human urine specimen, no parent 25I-NBOMe was detected. Following enzymatic hydrolysis of the glucuronides, four O-desmethyl metabolites were the only metabolites detected in this specimen.

Conclusion: The analysis of mouse hepatic microsomal preparations was an effective technique used to identify human urinary biomarker metabolites of 25I-NBOMe. 2-O-desmethyl-5-I-NBOMe appeared to be the most abundant of the metabolites detected in both of the human urine specimens.

This project was supported by the National Institute on Drug Abuse (NIDA) Center for Drug Abuse grant P30DA033934

Keywords: 25I-NBOMe, Metabolites, Hallucinogens

P82

Investigation and Validation of a 'Just Enough' Method for the Analysis of Drugs Used in Pain Management that Competes Effectively with Dilute and Shoot Methodologies

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Introduction: The 'Just Enough' method is a description of simple techniques such as filtration, protein precipitation, supported liquid extraction, extraction partitioning for toxicology screening. All Just Enough sample preparation techniques have the following features in common: 1) extremely easy to use; 2) minimal method development; 3) minimal time required; 4) very reproducible results; and 5) low relative standard deviations (RSDs).

The techniques used in the Just Enough types of analyses are compared to a typical 'Dilute and Shoot' method, which is an example of not incorporating sample preparation. The advantage of Dilute and Shoot is that it is very quick, only requiring the dilution of the sample (typically urine); therefore, a simple pipette to a fully automated instrument can accomplish this task. However, Dilute and Shoot is not a true sample preparation technique and involves only a dilution of the urine matrix and does not remove matrix interferences. In time, interferences will likely build up in the LC-MS/MS system inlet and HPLC column causing chromatographic problems such as carryover, loss of sensitivity and ion suppression.

Objective: Dilute and Shoot has been regarded by us to be a substandard method for the analysis of prescription drugs and drugs of abuse in urine by LC-MS/MS. Consequently, we investigated and validated a Just Enough method that competes effectively with the speediness and cost savings of a typical Dilute and Shoot LC-MS/MS method for urine, but drastically minimizes the problems related to excluding or bypassing sample preparation. This method was applied to 51 drugs that are typically analyzed in urine from pain management and/or drug rehabilitation programs.

Method: Our method combines 200 mcL of urine with 40 mcL of IMCS enzyme, 50 mcL of IMCS buffer and 5 mcL of the Internal Standard Mix using a Tecan Freedom EVO 100 liquid handler. For a 96-well plate, this process requires 8 min. The plate then incubated at 55°C for 35 min, which is then added to a 200 mcL MCA WAX (3mg) extraction tips from DPX, washed with 100 mcL of water, and eluted with 150 mcL 4% formic acid in MeOH (8 min). The total prep time is thus 51 minutes. The validation of this method exhibited excellent recoveries and RSDs for all 51 analytes.

Conclusion: A urine 96-well plate can thus be extracted, hydrolyzed and made ready for analysis by LC-MS/MS in under 1 hour. In comparison to a published Dilute and Shoot urine method, the time for dilution, hydrolysis, centrifugation and ready for injection was approximately 2 times as long. The difference in cost for materials between the two methods, except for the extraction tips, was virtually the same. However, as demonstrated by the method, the cost of the extraction tips is balanced by the significant savings in time, manpower and in instrument maintenance. Furthermore, our method has the advantage of highly increased sensitivity and much less concern for interferences that are inherent in a typical Dilute and Shoot LC-MS/MS urine method.

Keywords: Just Enough Methodology, IMCS Enzyme, DPX Extraction Tips, Urine Dilute and Shoot

P83

The Syntheses of the 2-O-desmethyl and 5-O-desmethyl Metabolites of 25I-NBOMe and Their Separation Using Hydrophilic Interaction Chromatography on a UPLC-MS/MS System

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Introduction: Recently, a new class of designer hallucinogenic drugs, dimethoxyphenyl-N-[(2-methoxyphenyl) methyl] ethanamine (NBOMe) derivatives, has become available via the Internet. Currently, 2-(4-iodo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl] ethanamine (25I-NBOMe) is the most popular abused derivative in the United States. It has been suggested that the three single O-desmethylated-25I-NBOMe metabolites present as glucuronidated conjugates, and 2,5-dimethoxyy-4-iodophenethylamine (2C-I) are major urinary metabolites of 25I-NBOMe. Two of these potential metabolites, 2-iodo-4-methoxy-5-[2-[(2-methoxyphenyl) methylamino]ethyl]phenol (2-O-desmethyl-5-I-NBOMe) and 5-iodo-4-methoxy-2-[2-[(2-methoxyphenyl)methylamino]ethyl]phenol (5-O-desmethyl-2-I-NBOMe), are not commercially available. The structure of these two metabolites was proved through syntheses and will be presented. We present and proved the structure of these two metabolites through syntheses. Also presented is the chromatographic separation of these metabolites from the other major 25I-NBOMe metabolites including 2-((2-(4-iodo-2,5-dimethoxyphenyl) ethylamino)phenol (25I-NBOH), 2C-I and 25I-NBOMe using hydrophilic interaction chromatography (HILIC) technique on an ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) system.

Objective: To provide methods of syntheses for 2-O-desmethyl-5-I-NBOMe and 5-O-desmethyl-2-I-NBOMe and UPLC chromatographic separation of these and other potential biomarker metabolites of the designer hallucinogen 25I-NBOMe.

Method: The syntheses of 2-O-desmethyl-5-I-NBOMe and 5-O-desmethyl-2-I-NBOMe were based on the previously published syntheses for 25I-NBOMe. The syntheses of 2-O-desmethyl-5-I-NBOMe involved an eight step process and the syntheses of 5-O-desmethyl-2-I-NBOMe involved a nine step process. The structure and purity of the resulting products was confirmed by NMR and mass spectrometry analysis. The chromatographic separation of these and the other potential biomarker metabolites of the designer hallucinogen 25I-NBOMe was performed using a Waters Acquity® TQD tandem quadrupole mass spectrometer with an UltraPerformance LC® controlled by MassLynx software. A United Chemical Technologies Selectra® PFPP column, 10 cm x 2.1 mm, 3.0 µm with the mobile phase consisting of A: water with 10mM ammonium formate and B: 90% acetonitrle contaning10% 10 mM ammonium formate at pH 6.8 was used. The following gradient allowed for the separation of 25I-NBOMe metabolites: 0.0-0.2min starting at 100% B, with a linear gradient to 50% B, and then returning at 4.5 min to 100% B. An injection volume of 5 µL was used with a mobile phase flow rate of 0.6 mL/min and a total run time of 5.0 min. The acquisition mode used was multiple reaction monitoring (MRM). The following transition ions (*m/z*) were monitored in MRM mode: 2-O-desmethyl-5-I-NBOMe and 5-O-desmethyl-2-I-NBOMe; 414>121 and 414>91, 25I-NBOH; 414>107 and 414>291; 2C-I; 308>91, 308>276 and 25I-NBOMe; 428>121, 428>91.

Result: Both 2-O-desmethyl-5-I-NBOMe and 5-O-desmethyl-2-I-NBOMe were successfully synthesized by the presented methods. Both of these compounds were determined to be of 97% purity The HILIC method presented for the analysis of 2-O-desmethyl-5-I-NBOMe, 5-O-desmethyl-2-I-NBOMe, 25I-NBOH, 2C-I and 25I-NBOMd resulted in separation of these potential urinary biomarkers.

Conclusion: The ability to synthesize the commercially unavailable 25I-NBOMe metabolites, 2-O-desmethyl-5-I-NBOMe and 5-O-desmethyl-2-I-NBOMe and to separate them chromatographically on an UPLC-MS/MS instrument from other 25I-NBOMe metabolites allows for the confirmation and identification of theses potential biomarkers in human urine specimens where 25I-NBOMe use or exposure is suspected.

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Keywords: Syntheses, 25I-NBOMe, Metabolites, Hydrophilic Interaction Chromatography

P84

Evaluation of NBOMe Derivatives, 25I-NBOMe, 25B-NBOMe, 25C-NBOMe and 25I-NBOH Refrigerated Stability in Four Types of Blood Collection Tubes Over a 72 Hour Period

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Introduction: Recently, a new class of designer hallucinogenic drugs, dimethoxyphenyl-N-[(2-methoxyphenyl) methyl]ethanamine) (NBOMe) derivatives, have become widely available via the Internet. For the past four years, our laboratory has been identifying and quantifying NBOMe drugs in serum and urine specimens obtained from intoxicated patients from across the country and around the world. Often, blood samples are collected in tubes containing serum or plasma separators when patients are admitted to an emergency room after apparent drug overdose. These specimens often sit in the collection tubes for days prior to or during shipping. It is known that serum separator tubes can cause lower serum concentrations of some drugs such as tricyclic antidepressants. The stability of NBOMes in various blood collection tubes has yet to be investigated.

Objective: To determine refrigerated NBOMe stability, or lack thereof, in various popular blood collection tubes of the presently Schedule I 25I-NBOMe, 25C-NBOMe, 25B-NBOMe and 25I-NBOH. The results of this study will provide valuable guidance in the collection and transportation of serum specimens potentially containing NBOMe-related compounds.

Method: Each drug was added to drug free serum at 1ng/mL, then pipetted into each of the following collection tubes: Red Top (366431) BD Vacutainer® glass serum tube with conventional closure; Gray Top (367921) BD Vacutainer® Plus plastic sterile tube with grey BD Hemogard™ closure containing sodium fluoride, 5.0 mg and potassium oxalate, 4.0 mg; Gold Top (367977) BD Vacutainer® Plus plastic SST™ serum tube with gold BD Hemogard™ closure containing clot activator and gel for serum separation and silicone coated interior; and Green Top (367962) BD Vacutainer® PST™ Tubes containing spray-coated lithium heparin and a gel for plasma separation. The tubes were then inverted three times and placed in the refrigerator. Three aliquots of serum from each tube were then removed at 1, 4, 24, and 72 hours post-preparation, extracted using Clean Screen ZSDUA020 SPE extraction columns as described by Poklis et al (Biomed. *Chromatog. 2013*), and quantified using a Shimadzu SCL HPLC system with an Applied Biosystems 3200 Q trap. The observed r^2 values for the NBOMe calibration curves were 0.990 or better. The LOQ was administratively set at 25pg/mL. Validation criteria for quality control specimens as well as matrix effects, absolute recovery, carryover and specificity met acceptable validation criteria.

Result: The Grey Top tubes showed no significant drug loss for any of the four NBOMe derivatives over the 72hr time period. The Gold Top tubes with serum separator and Green Top tube with plasma separator demonstrated a significant lose in all four drugs after 24hr with a typical drug loss of 15% to 25%. 25B-NBOMe had the greatest losses at 72 hours of 49% and 48% in Green Top and Yellow Top tubes, respectively. Over the same time, 25C-NBOMe showed the least loss, 18%. Surprisingly, Red Top tubes showed significant drug loss for all NBOMe derivatives over the 72hr time period.

Conclusion: This study demonstrated that all four of the NBOMes degrade or are sequestered over time in Gold Top serum or Green Top plasma separator blood collection tubes. There should be a statement here about the red top tube. Serum should be collected in grey top tubes for NBOMe derivatives analysis.

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Keywords: 25I-NBOMe, 25B-NBOMe, 25C-NBOMe, 25I-NBOH, Blood Collection Tubes, Serum Separator Tubes, Plasma Separator Tubes

P85
Quantitative Measurement of Dextromethorphan in Oral Fluid by LC-MS/MS

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Background: Dextromethorphan (DXM) is an antitussive drug found in commonly used non-prescription cold and cough medications. At lower doses, DXM is a safe drug that does not produce adverse reactions. However, abuse of DXM at higher doses has been reported among adolescents and young adults. According to the data collected by the National Poison Data System during 2000-2010, 44,206 cases of DXM abuse have been reported. There are case reports to support the withdrawal and the dependence syndrome of DXM as well. DXM is consumed orally and is absorbed by the gastrointestinal tract. DXM is metabolized in the liver to its O-demethylated metabolite, dextrorphan (DXO). Pharmacologically, the mechanism of action of DXM and DXO are N-methyl-D-aspartate agonists, producing psychological effects similar to those of ketamine and phencyclidine (PCP) at high doses. Presence of DXM and DXO in oral fluid has been reported. One of the arguments posed against oral fluid drug testing is the contamination of the collection pad with residual drugs in the mouth. The detection of the drug metabolites in oral fluid provides defense against this argument and it also minimizes the concerns of passive exposure.

Objective: To develop a rapid and sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for quantitative measurement of DXM and its metabolite, DXO, in oral fluid.

Method: Oral fluid samples were collected with Quantisal™ (Immunalysis) collection devices, which consist of a cotton pad and an extraction buffer. Under the routine collection procedure, the cotton pad is placed in the mouth to absorb 1 mL of oral fluid. Because original oral fluid was diluted with extraction buffer during the collection, 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. Only 400 µL of the collected sample were used for the SPE. Samples were screened with a commercially available ELISA method prior to LC-MS/MS analysis.

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). Run time was 4 minutes. Electrospray ionization mass spectrometry was performed on a TQD instrument (Waters). Analysis was performed in positive ionization (ESI+) and multiple reaction monitoring (MRM) mode. Two transitions, a quantifier and a qualifier, were used to identify the target analytes. MRM transitions for DXM were 272.3>147.0 m/z and 272.3>171.1 m/z and MRM transitions for DXO were 258.0>157.0 m/z and 258.0>133.1 m/z.

Result: The developed method was validated according to Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines. The linear dynamic range was 5-100 ng/ml with a lowest limit of quantitation (LLOQ) of 5.0 ng/ml for DXM and DXO. Overall, the results of the accuracy and the precision values were within the acceptance criteria for both drugs. However, the intra-day precision of DXO at 5 ng/mL was 21.2, and that was outside the acceptance criterion for LLOQ. In addition, selectivity, matrix effect and recovery were calculated for the LC-MS/MS method.

Fifty nine authentic samples that include previously tested positives and negatives were tested with the current method to evaluate the applicability of the method. Out of 59 samples tested, thirty samples tested positive for DXM or DXO. Twenty-eight samples had both DXM and DXO. Two samples had only DXM.

Keywords: Dextromethorphan, Dextrorphan, LC-MS/MS, Oral Fluid

P86

Biochip Array Technology Immunoassay Performance and Quantitative Confirmation of Designer Piperazines for US Military Urine Workplace Drug Testing

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Introduction: Designer piperazines are emerging novel psychoactive substances (NPS) with few high-throughput screening methods for their identification.

Method: We evaluated a biochip array technology (BAT) immunoassay for phenylpiperazines (PNP) and benzylpiperazines (BZP), and analyzed 20,017 randomly collected anonymized US military urine workplace specimens. Urine specimens initially screened negative for amphetamines, cannabinoids, cocaine, opiates and phencyclidine; and were stored at room temperature between 10 to 233 days prior to BAT immunoassay analysis. Immunoassay performance with three specific antibodies at recommended cutoffs was evaluated for PNPI (5 µg/L), PNPII (7.5 µg/L), and BZP (5 µg/L). Eight-hundred forty presumptive positive designer piperazines and 206 randomly selected presumptive negative specimens were confirmed by liquid chromatography high-resolution mass spectrometry (LC-HRMS) for the presence of eight piperazines and/or trazodone. Additional stability studies were performed extending storage up to four weeks at room temperature, 4°C and -20°C to evaluate piperazine and trazodone instability.

Result: Assay limits of detection for PNPI, PNPII and BZP were 2.9, 6.3 and 2.1 µg/L, respectively. Calibration curves were linear (R^2 >0.99) with upper limits of 42 µg/L for PNPI/PNII and 100 µg/L for BZP. Quality control samples demonstrated imprecision <19.3%CV and accuracies 86.0-94.5% of target. There were no interferences from 106 non-piperazine substances. From 840 presumptive positive specimens, 78 (9.3%) were confirmed LC-HRMS positive for piperazines, with 72 of these concurrently positive for trazodone (4.2-4218 µg/L) and its metabolite, 1-(3-chlorophenyl)piperazine (mCPP) at 2.5-2277 µg/L. Additionally, four presumptive positive specimens contained BZP (3.1-179 µg/L), while 1 contained 189 µg/L BZP and 22.4 µg/L 1-(3-trifluoromethyl)piperazine (TFMPP). Only one presumptive positive specimen confirmed positive for 2.6 µg/L mCPP only. Only trazodone, an antidepressant commonly prescribed to treat post-traumatic stress disorder, was detected in 13 presumptive positive specimens (2.7-869 µg/L). Only two false negative specimens, containing mCPP (3.3 µg/L) or BZP (3.6 µg/L), were detected from 206 presumptive negative specimens. BAT specificity (21.1 to 91.4%) and efficiency (27.0 to 91.6%) increased, and sensitivity slightly decreased (97.5 to 93.8%) with optimized cutoffs of 25 µg/L PNPI, 42 µg/L PNPII, and 100 µg/L BZP. However, PNPII and BZP optimized cutoffs also were at the immunoassay's upper limit of linearity. For all storage conditions, piperazines were unstable (>20% analyte loss) after 4 weeks except for TFMPP (≤17% loss), while trazodone showed minimal loss (≤6%). The prevalence of piperazines in this population was 0.4%, but possibly was underestimated due to instability of these analytes at room temperature.

Conclusion: A high-throughput screening method is needed to identify piperazine NPS intake. We documented improved performance of the Randox BAT immunoassay when antibody cutoffs were optimized based on LC-HRMS analysis. In addition, in anonymized US military workplace urine drug tests, all but two positive specimens contained mCPP and/or trazodone, most likely from legitimate medical prescriptions.

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Keywords: Piperazines, HRMS, Biochip

P87 ***"NIJ Funded"***

Amplified Colorimetric Detection of Cocaine in Oral Fluid Based on Exonuclease-Assisted Aptamer Strand Recycling

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Background: Cocaine is a powerful psychoactive and addictive central nervous system stimulant. The detection of cocaine is a significant challenge for public health authorities and law enforcement agencies. A rapid screening for cocaine has been usually achieved by competitive immunoassays. Unfortunately, these immunoassays have inherent disadvantages from the employed antibodies such as, poor specificities and batch-to-batch variations. Aptamers are single-stranded oligonucleotides selected *in vitro* by Systematic Evolution of Ligands by EXponential enrichment (SELEX). They bind to a broad range of targets with specificity superior to antibodies, exhibiting immense potential in the biosensor field. The aptamer-based sensors have achieved excellent specificity; however, previous cocaine sensors have demonstrated the limited sensitivity due to their low binding affinity of the aptamer to cocaine.

Objective: To improve the sensitivity of the aptamer-based cocaine sensor, we here use Exonuclease III (Exo III)-Assisted Aptamer Strand Recycling (EAASR) strategy to colorimetrically amplify the cocaine-binding event through the aggregation of DNA-modified Au nanoparticles (AuNPs), performing rapid and sensitive detection of cocaine in oral fluid at room temperature.

Method/Result: The AuNP-based color change was used for the detection of cocaine in oral fluid. We have discovered that Exo III rapidly digested the cocaine-binding aptamer in the absence of cocaine while the Exo III digestion of aptamer was significantly inhibited when the aptamer was bound to cocaine. Based on this finding, we used the complementary DNA (cDNA)-modified AuNPs to hybridize/recycle the cocaine-bound aptamer strands (the process termed Exonuclease III (Exo III)-Assisted Aptamer Strand Recycling (EAASR)) to amplify the cocaine-binding event and sensitively detect low concentration of cocaine in sample. Specifically, in the presence of cocaine, the cocaine-bound aptamers hybridized with the cDNA probes modified on the AuNPs to form the duplexes. Exo III then recognized the surface-bound duplex, selectively digesting the cDNA probe. Once the cDNA probe was digested, the aptamer strand was released and recycled to begin the cycle anew. When all cDNA probes were digested from the surface, the AuNPs lost their stability and aggregated, forming a visible red-to-blue color change. In the absence of cocaine, all aptamers were digested and the AuNP-conjugated cDNA probes did not get recognized/digested by Exo III due to their single-stranded form. The solution remained red. In order to examine the specificity of our sensor, we prepared a sample set consisting of oral fluid samples that were fortified after collection with various concentrations of cocaine, cocaine interferences such as ecgonine and benzoylecgonine. These samples were analyzed using a double blind approach. Results were then compared with existing immunoassays such as NEOGEN cocaine/benzoylecgonine ELISA kit.

Conclusion: As a result, cocaine concentrations as low as 300 ng/mL in oral fluid can be readily observed by naked eye within 30 min at room temperature. Compared to competitive immunoassays, our sensor showed consistent results in detecting cocaine and demonstrated better specificity against cocaine major metabolites, resulting in zero cross reactivity.

Keywords: Aptamer, Cocaine, Exonuclease III (Exo III)-Assisted Aptamer Strand Recycling

P88 ***"NIJ Funded"***

Analysis of Heroin and Methamphetamine in Human Hair: Surface Contamination and Localization of Analytes

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Introduction: For more than two decades, researchers and scientists have investigated and employed hair testing for drugs of abuse as an alternate matrix to blood and urine. Despite considerable research, given current analytical technologies and interpretive methods, environmental contamination remains an unresolved issue for hair testing, and controversy exists over the source of drug residues found in hair and the potential for environmental contamination to cause false-positive test results.

Objective: The purpose of this study was to 1) examine the impact of environmental contamination on drug tests conducted with human hair, 2) examine the effectiveness of a hair decontamination procedureused by forensic laboratories, and 3) look for markers to distinguish between contamination and ingestion.

Method: Methamphetamine (MAMP) and heroin were applied to human hair (8 mg drug per 10 g hair) by dry transfer. Both in vitro contaminated non-drug user hair and drug user hair were shampooed regularly for 8 weeks. Samples were taken at multiple time points and analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) with and without decontamination. We monitored the levels of the parent drugs, as well as the known metabolites amphetamine (AMP), 6-acetylmorphine (6-AM), and morphine. A mixed model that accounts for repeated measures and clustering was used to analyze the data. The model included the following direct effects: type of hair (drug user hair, contaminated hair), decontamination, hygiene (shampooed and not-shampooed), time, as well as second, third and fourth interactions. Contrasts were used to investigate the significance of treatment interactions at different time points.

In addition, we used several imaging techniques to attempt to localize the site of incorporation of drugs into hair as an indicator of the route of incorporation (e.g., contamination versus consumption). These techniques included matrix assisted laser desorption ionization–time of flight (MALDI-TOF) MS, atomic force microscopy-infrared spectroscopy (AFM-IR), time-of-flight secondary ion MS (TOF-SIMS) and scanning electron microscopy with electron dispersive x-ray spectroscopy (SEM-EDS).

Result: After contamination with MAMP and heroin, detectable levels of the metabolites AMP, 6-AM, and morphine were found. Levels of AMP and morphine in contaminated hair that was not shampooed increased over the 8 week time period of the study in externally contaminated hair, whereas there was no detectable change in the concentrations of these analytes in the user hair.

The decontamination procedure reduced the levels of all analytes of interest in both user and externally contaminated hair ($p < .03$). In externally contaminated hair, after 8 weeks of shampooing and laboratory decontamination the concentrations of analyte remaining on the hair (non-decontaminated values in parenthesis) were: heroin .02 (.02), 6-AM .09 (0.13), morphine .06 (.09), MAMP, 2.65 (4.35), AMP .02 (.03) ng/mg.

MALDI-TOF has sufficient sensitivity to detect the analytes of interest in drug user and externally contaminated hair, but lacks the spatial resolution needed to determine the site of incorporation of the drug molecules. The other imaging techniques had much better spatial resolution, but due to the small region being sampled, did not have enough sensitivity to detect the analytes of interest in any of the user or externally contaminated hair samples.

Conclusion: Environmental contamination by methamphetamine and heroin may be a significant confounder to interpreting hair-testing results. In currently available imaging techniques the increased spatial resolution needed to localize the presence of analytes of interest to a particular region of the hair results in a lack of sensitivity to detect the analytes at physiologically relevant concentrations.

Keywords: Hair, Methamphetamine, Contamination

P89
Tissue Distribution Following a Fatal Diphenhydramine Intoxication

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Introduction: A 35-year-old female was found minimally responsive on the floor with vomitus next to her head. Blood pressure was 107/57 and she experienced a seizure during transport to the hospital. Past medical history indicated chronic pain of undetermined origin and depression. Depression was treated with multiple medications including venlafaxine and alprazolam. Search of the decedent's room revealed three empty bottles of "sleeping pills" with each bottle contents listed as sixty 15 mg pills (total 2700 mg). The patient was unresponsive upon presentation to the emergency room with signs of anticholinergic syndrome including severe metabolic acidosis, dilated pupils, increased body temperature and an electrocardiogram tracing consistent with widened QRS complex. Supportive care was withdrawn following a brain death diagnosis within 24 h of admission. Examination was conducted three days after the decedent was declared dead and revealed a cerebral infarction, congested lungs and edema.

Objective: To determine the tissue and fluid distribution of relevant drugs and metabolites.

Method: Specimens were analyzed for volatiles by headspace gas chromatography (GC)/flame ionization detection. Antemortem urine and postmortem heart blood were screened by immunoassay. Alkaline-extractable drugs were analyzed by GC/full scan mass spectrometry (MS). Analytes were confirmed by GC/selected ion monitoring mode MS. Gastric contents and tissues were diluted with deionized water at ratios that allowed for quantification within the dynamic range. Urine, blood and vitreous were extracted without additional sample preparation.

Result: GC/MS analysis confirmed alprazolam, α-hydroxyalprazolam and oxymorphone in urine and 0.08 mg/L diazepam, 0.06 mg/L midazolam and 0.05 mg/L alprazolam in antemortem (AM) blood. Diphenhydramine, venlafaxine and *O*-desmethylvenlafaxine were confirmed in postmortem (PM) heart blood. Diphenhydramine (DPH), venlafaxine (VEN) and *O*-desmethylvenlafaxine (ODV) concentrations (mg/L or mg/kg) are summarized below.

	AM Blood	**PM Heart Blood**	**Vitreous**	**Bile**	**Gastric**	**Liver**	**Heart**	**Kidney**	**Lung**	**Spleen**	**Brain**
DPH	12.40	15.00	8.43	141.01	145.59	149.84	33.96	87.79	14.89	76.52	53.78
VEN	0.13	1.26	0.34	1.56	154.29	11.99	4.04	4.43	4.14	5.51	2.88
ODV	0.23	0.35	0.29	1.64	3.19	5.56	1.53	2.79	1.90	0.74	1.84

Conclusion: To our knowledge, this study presents the most comprehensive diphenhydramine tissue distribution following a fatal intoxication. Overall, diphenhydramine concentrations were within published lethal ranges. Therapeutic concentrations were present for all other medications. Cause of death was ruled diphenhydramine intoxication. Diphenhydramine was previously reported to inhibit *in vitro* and *in vivo* CYP2D6-catalyzed venlafaxine metabolism to *O*-desmethylvenlafaxine. In this case, it was inconclusive whether elevated diphenhydramine concentrations affected venlafaxine disposition.

Keywords: Postmortem, Tissue Distribution, Diphenhydramine, Venlafaxine

P90

Ultra-Rapid Analysis Method of Flakka (alpha-PVP), a Synthetic Cathinone Drug, in Urine and Serum at 9 Seconds per Sample by Laser Diode Thermal Desorption Mass Spectrometry

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Background/Introduction: In April 2015, NIH published that the emerging drug alpha-pyrrolidinovalerophenone (alpha-PVP), or popularly known as "Flakka", is surging in Florida and in other parts of the US. It is structurally related to pyrovalerone, a psychoactive drug that was used to treat chronic lethargy and fatigue, which has amphetamine life effects. Alpha-PVP is widely sold on the internet as a "research chemical", a "legal high" or "bath salts" probably with the intention of using it as a recreational drug as legal substitutes for illicit compounds such as MDPV.

Objective: A comprehensive detection and quantification method for the analysis of emerging drugs of abuse in biological matrices is needed. We developed an ultra-rapid, high-throughput and cost effective analytical method for the detection of alpha-PVP using the LDTD coupled to an API-5500 Qtrap system. Method development and validation were performed in urine and serum matrices and the analysis time needed was 9 seconds per sample.

Method: Urine and serum were spiked with alpha-PVP at concentrations ranging from 2 to 200 ng/mL forming calibration curves. The extraction procedure consisted of using a 200 µL sample aliquot to which were added 20 µL of the internal standard (alpha-PVP-d8, 200 ng/mL) in methanol, 200 µL of sodium carbonate (0.5M at pH 10) and 400 µL of hexane:ethyl acetate (75:25, v/v). Samples were mixed. After phase separation, 100 µL of upper layer were transferred and 10 µL of HCl (0.01N in Methanol) were added and mixed. A 5 µL aliquot was transferred into a 96-well plate (LazWell-HDE) and evaporated to dryness. Samples were desorbed using a laser pattern consisting of a 6 second ramp going up to 65% laser power, holding for 2 seconds. The mass spectrometer was operated in MRM mode and all the compounds were simultaneously measured in the extracts. The quantification transition used was m/z 232.1→126 and confirmation transition was m/z 232.1→91. The ionization was performed in APCI positive mode. The following validation parameters were evaluated: Linearity, intra-run and inter-run accuracy/precision, extracted sample stability, drug interference (cross reactivity assay) and matrix effect.

Result: Calibration curves had excellent linearity with r^2 values between 0.9961 and 0.9979 in urine and serum respectively. The intra-run accuracy and precision across the calibration curves were between 91 and 112% and 0.3 and 6.9%. Following the extraction procedure, all samples were stored at 4°C to evaluate the drugs temporal stability in wet state. After a waiting period, all samples were re-spotted and analyzed. A wet stability greater than 16h was obtained with accuracy and precision of 97.7% and 9.9% for concentrations equivalent to the LOD, respectively. 32 different (Amphetamines, Benzodiazepines, Opiates, Cannabinoids and Hallucinogens) drugs were tested at a final concentration of 1 µg/mL for the cross reactivity assay. No interference was obtained for all drugs. Matrix effect was verified using 10 different real matrices. Basal concentration was evaluated and then a known quantity of alpha-PVP was spiked at QC med level to verify the matrix effect. QC concentrations were within the 15% nominal concentration of the spiked value.

Conclusion/Discussion: The LDTD technology provides a selective, sensitive and ultra-rapid analysis method, i.e. 9 seconds per sample, for the detection and quantification of alpha-PVP drugs in urine and serum samples at concentrations ranging between 2 and 200 ng/mL.

Keywords: High Throughput, LDTD-MS/MS, Alpha-PVP

P91

Determination of CB_1 Receptor Agonist Activity of Emerging Synthetic Cannabinoids in GH4C1 Cells

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Introduction: The United State Congress passed The Synthetic Drug Abuse Prevention Act on July 9, 2012. This legislation places synthetic cannabinoids into Schedule I of the Controlled Substances Act (21 U.S.C. 812(c)) based on structure, receptor binding, and function. There are currently at least 17 chemical classes of cannabinoid structure known with all but two reported in the literature in seized samples prior to 2015. A number of these reported cannabinoids have cores or substitutions that fall outside of the definitions of an analog under current regulations and it is unknown if they are cannabinoid receptor 1 (CB_1) agonists.

Objective: Assess the binding affinity and activity of new synthetic cannabinoid compounds at the CB_1 receptor.

Method: Using a cell-based cAMP assay (Amersham), we have demonstrated the ability to collect data pertaining to cell signaling and functionality of the CB_1 receptor. Using the cell-line GH4C1, known to express the CB_1 receptor, we measured changes in forskolin-induced cAMP production in cells treated with a potent CB_1 receptor agonist (CP-55,940) using an EIA method to measure cAMP levels through the displacement of cAMP peroxidase. GH4C1 cells were treated with 3.0 μM forskolin immediately followed by 0.2 to 8.0 nM CP-55,940 for 10 min. The experiment was repeated adding increasing concentrations of the CB_1 receptor antagonist rimonabant (1 to 100 nM). The end product was recorded using a plate reader (Biotek Synergy 2) at 650 nm and data presented as percentage bound for each standard and sample (%B/B_0).

Result: Preliminary data demonstrated a dose-dependent decrease in forskolin induced cAMP production in GH4C1 cells (approximately 60 to 100%) following treatment with CP-55,940. Further, this increase in forskolin induced cAMP production was reversed by the introduction of a CB_1 receptor antagonist.

Discussion/Conclusion: We have demonstrated the ability to measure the binding affinity and activity of synthetic cannabinoid compounds specific to the CB1 receptor. Future research will include further method optimization with the inclusion of LC/MS/MS methods and determination of EC_{50} values for a range of synthetic cannabinoids. The resulting analytical data and methodologies will help advance forensic chemistry and toxicology by providing data to fill the gap in research needed to classify a synthetic compound as a CB_1 receptor agonist. The goals of this research also in part aid in placing these dangerous family of synthetics into the Schedule 1 category of the Controlled Substances Act.

Keywords: Synthetic Cannabinoid, Emerging Drugs, CB_1 Agonist

P92

Determination of Synthetic Hallucinogens: 25I-, 25C-, and 25B-NBOMe by LC/MS/MS

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Introduction: Among the drug using population, the use of designer compounds has increased over the past decade. An emerging synthetic LSD compound (NBOMe) has joined the popular designer compounds such as cathinones (bath salts) and synthetic cannabinoids (JWH). Recently, the DEA classified 25I-, 25B-, and 25C-NBOMe as *schedule I* drugs (2013). In postmortem cases, the average concentrations of NBOMe drugs encountered were less than 0.5 ng/mL in blood and urine specimens. Encountering lower concentrations increases the need for a sensitive method to determine NBOMe drugs in biological samples.

Objective: Development of a sensitive, reliable and reproducible method for quantification of NBOMe drugs in blood and urine using deuterated 25I-NBOMe.

Method: Negative blood and urine samples were spiked with known concentrations of standards and D3-25I-NBOMe as internal standard (IS). Samples were extracted with organic solvent under basic conditions, evaporated, reconstituted and analyzed on Agilent 1260 Infinity series LC system coupled with 6460 QQQ with JetStream Technology and ESI source. Chromatographic separation was achieved on a C-18 column with gradient elution. Mobile phases of water:methanol (90:10 v/v) with 5 mM ammonium formate (solvent A) and acetonitrile with 0.1% formic acid (solvent B) were used in gradient elution program; 30% B to 70% B over in 3 mins, returning to initial 30% of B over in 0.5 mins and held for 0.5 min for a total run time of 4 min. Data was acquired on positive mode, MRM transitions monitored for 25I (428– 121/91 m/z); 25B (382– 121/91 m/z); and 25C (336– 121/91 m/z).

Result: New ion transitions for the IS were identified (431 – 121.1/ 91.5) and used in the quantification of all three compounds. The quantification method was validated for precision and accuracy (n=5). All three compounds demonstrated good linearity over a concentration range of 0.005 ng/mL to 10 ng/ mL for both blood ($r^2 \geq 0.997$) and urine ($r^2 \geq 0.999$); LOD and LOQ were established as 0.005 ng/mL for all three compounds. Analytical recoveries were between 85.6% and 96.4%. No interferences were observed from the classical illicit drugs, JWH compounds, LSD, ketamine, fentanyl, and 2-CE.

Conclusion: The extraction and LC/MS/MS method developed for analysis of blood and urine for 25I-, 25B-, and 25C-NBOMe is precise, sensitive and reproducible at forensically relevant concentrations.

Keywords: 25I-NBOMe, Synthetic LSD, D3-25I-NBOMe

P93

Combining Complimentary Ion Ratio and Library Matching Confirmatory Techniques in One LC-MS/MS Method

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Introduction: Full scan MS/MS library searching results have shown to be an effective confirmation technique that allows confidence in compound identifications. The LC-MS/MS technique allows the generation of a chemical fingerprint for a compound through the acquisition of a product ion spectrum. The acquired product ion spectrum is then searched against an MS/MS spectral library for compound matching.
QTRAP technology enhances this workflow by trapping and accumulating product ions and producing a more enhanced and better quality product ion spectrum (EPI) than can be produced on a traditional triple quadrupole instrument. A QTRAP instrument can be utilized in multiple ways in the same experiment. As well as performing the library confirmation workflow, quantification can be performed by using Multiple Reaction Monitoring (MRM) as the survey MS scan for compound detection and triggering the full scan product ion scan in an information dependent acquisition (IDA). The MRM is the gold standard for quantification due to the most sensitive and selective form of mass spectrometry. In many labs two MRM transitions are monitored and the ion ratio is used for confirmation purposes. Quite often interfering compounds will distort the ion ratio, potentially causing a false negative result. This reliance on ion ratio can be alleviated if a library spectral searching match is obtained from the same analysis. On the other hand, some compounds, namely the barbiturates, don't break apart very well in a tandem MS experiment in which to produce a sufficiently enough specific product ion experiment in library searching.

Objective: We aimed to combine the ion ratio with library searching confirmatory techniques as a complimentary workflow but achievable in one injection. The objective was to develop a polarity switching IDA triggered EPI method to allow for high-quality screening of over 100 target analytes in 10 minutes.

Method: The LC-MS/MS method consisted of a Shimadzu Prominence XR and SCIEX 4500QTRAP LC/MS/MS System. LC separation was achieved on a Phenomenex Kinetex C18 (3.0 x 50mm, 2.6 u) column using a 10 minute gradient. The MS method employed an MRM-/MRM+–IDA–EPI approach set up to monitor two MRMs for the compounds in negative mode and only one MRM per compound in positive mode but to trigger a full scan product ion spectrum for every detected compound in positive mode only. All acquired product ion spectra were searched against a forensic MS/MS mass spectral library for compound identification from the positive mode and the negative mode compounds were confirmed using the traditional ion ratio approach. Urine samples were diluted 10-fold in mobile phase A, centrifuged and10 µL sample was injected.

Result: In the 10 minute LC run time most critical compound separations were achievable, namely morphine/hydromorphone; methamphetamine/phentermine. Separation was not achieved for Quinine/quinidine and Amobarbital/pentobarbital. The MS method successfully triggered a product ion for all the positive mode targeted analytes; all compounds had library match scores >75%. MRM ratio variability results for the negative mode compounds included a Peak area %CV for all transitions of <18% (n=15) and MRM Ratio of <23%CV for all analytes.

Conclusion: A polarity switching IDA triggered EPI method has been developed which allows for high-quality screening and confident identification of 105 target analytes in 10 minutes.

Keywords: LC-MS/MS, Hybrid Linear Ion Trap, Confirmatory Techniques

P94

Investigating the Enhancement in Selectivity for the Analysis of Methyldienolone in Urine Samples by Differential Mobility Spectrometry

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Introduction: One of the most common issues in LC-MS/MS method development is the presence of isobaric matrix interferences that makes integrating chromatographic peaks and reaching required detection levels challenging. Commonly these interferences are removed by either adjusting HPLC conditions or by modifying sample preparation. These approaches however increase both method development time and the final method result turnaround times. Differential mobility spectrometry (DMS) has the potential to resolve these interfering compounds during the LC-MS/MS run enabling minimal sample preparation and reduced LC run times. Differential mobility spectrometry is a method of separating ions based on difference between their ion mobility's in a high and low electric field in gases at or near atmospheric pressure. It is essentially used as ion-pre-filter for mass spectrometry. Here we investigated the use of an ion mobility cell to resolve isobaric interferences in the analysis of methyldienolone in human urine samples that cannot be achieved by tandem mass spectrometry alone.

Objective: We aimed to investigate the capabilities of a DMS device coupled with LC-MS/MS to provide a highly selective quantitation method for methyldienolone; separating isobaric matrix interferences from the target analyte, in a simple dilute and shoot workflow.

Method: The SCIEX QTRAP® 5500 was operated in Multiple Reaction Monitoring (MRM) mode. Electrospray Ionization was employed in positive polarity. Three selective MRM transitions (237.2 / 135.1; 237.2 / 91.0; 237.2/ 77.0) were monitored for methyldienolone. Gradient elution was performed through a Waters C18 column maintained at 60 °C with a flow rate of 500 µL/min. The injection volume was set to 10 uL. The DMS device was operated at the low temperature and low resolution settings without the use of modifier. Separation voltage and compensation voltage were set at 3000 V and 3.0 V respectively while the offset voltage was kept as -3.0 V. Urine samples were centrifuged, diluted 5 times and analyzed as per the dilute and shoot methodology.

Result: Isobaric interferences were found at the retention time of methyldienolone with the dilute and shoot LC-MS/MS method, based on MRM alone. Also, there was high baseline associated with the MRM transitions used for this analysis. By using the LC-DMS-MS/MS setup and selecting the appropriate value for compensation voltage these isobaric interferences and the high baseline were completely removed. A matrix matched calibration curve was generated from 0.1 ng/mL to 500 ng/mL. The regression co-efficient obtained for the linear plot was (r): 0.9956 which was generated by applying linear regression with a weighting factor of 1/X2. Spiked concentration at 0.5 ng/mL (1 pg on column) extracted from matrix sample was selected as the limit of quantitation with S/N> 70. The % CV intra-day precision (n=10) was 2.05 and inter-day precision was 3.2 (n=20). Average recovery at 0.5 ng/mL for urine was 101.6%.

Conclusion: The DMS technology was demonstrated to be successful in the optimization of a dilute and shoot methodology for the analysis of methyldienelone; allowing the use of this simplified sample preparation approach by eliminating all interfering ions. This in turn reduces the background noise and increasing the S/N value and therefore allows an improvement in detection limits and quantitative accuracy. The LOD of 0.05 ng/mL and LOQ of 0.5 ng/mL were achieved using the DMS, which were otherwise 2 and 5 ng/mL respectively without the DMS technology.

Keywords: Differential Mobility Spectrometry, Enhanced Selectivity, Simplified Sample Preparation

P95

Screening of Over 300 Compounds in Urine Using Triple Quadrupole Mass Spectrometer and Software for Rapid Data Analysis

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Introduction: Forensic toxicologists need tools for rapid and confident screening for large numbers of compounds. Triple quadrupole mass spectrometers are becoming the instruments of choice in many forensic toxicological laboratories; supplanting traditional immunoassays because they offer better specificity within compound classes, can test for a wider range of compounds, and can easily add more compounds to the test menu. In order to generate results as fast as immunoassays, mass spectrometry methods must contain a larger number of analytes in one analytical run. Additionally, software is needed for easy and rapid data analysis of the large panels.

Objective: Demonstrate a qualitative screening method for over 300 compounds on a triple quadrupole mass spectrometer with associated data analysis software. Compounds analyzed include opiates, opioids, benzodiazepines, barbiturates, amphetamines, tricyclic antidepressants, illicit compounds, and more.

Method: Samples were prepared either by urine dilution or enzymatic hydrolysis followed by further dilution; both schemes had a final dilution of 30-fold. Tolbutamide-d9 was used as a single internal standard. Samples were introduced to a Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer after gradient chromatographic separation. One to three SRM transitions in both positive and negative ionization mode were collected for over 300 analytes, depending on the specific fragmentation pattern for a particular compound. Data was acquired with Thermo Scientific TraceFinder™ software and analyzed with Thermo Scientific ToxFinder™ software. Screening performance was assessed by analyzing compounds in pools of ten each at a single concentration of 100 ng/mL. Pools of ten compounds were considered adequate as more than this is not usually found in a single sample. Additionally, 50 donor samples previously analyzed in a collaborator laboratory were also analyzed with this method. ToxFinder software identified compounds based on retention time, number of product ions detected and product ion ratios. Semi-quantitation can be performed either by using a single point calibrator or by using internal standard ratio

Result: Over 90% of the compounds in the spiked pooled samples tested screened positive for at least one SRM transition. Many of the negatively screened compounds have higher cutoff limits, and so it was not unexpected that they were not reported as positive. As expected, more compounds were reported as positive with one confirming SRM transition, with fewer compounds being reported as positive as the number of confirming SRM transitions increased. More confirming transitions offer better specificity, but come at the price of reduced sensitivity. Data for donor urine samples were consistent with the previous results from the collaborator laboratory.

Conclusion: We successfully screened for over 300 compounds. This combination of instrumentation and software are a fast and reliable platform for screening compounds in a forensic toxicological setting.

Keywords: Screening, Mass Spectrometry, Urine

P96 ***"NIJ Funded"***

The Importance of Evaluating Internal Standard Addition Methods in Dried Blood Spot Analysis

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Introduction: Dried blood spot (DBS) analysis is well-established in the area of clinical testing (e.g. neonatal screening). In forensic toxicology it has the potential to increase sample stability allowing samples to be stored for longer periods of time, create a safer work environment for sample collectors and analysts due to the small sample volumes and significantly reduce costs related to sample transportation. In most quantitative assays involving liquid samples, internal standard (ISTD) is added directly to the sample. In DBS analysis, this approach is not always possible (e.g. sample taken from finger and spotted directly onto card) and makes the addition of ISTD a challenge. It is common practice in clinical DBS applications to add ISTD to the extraction solution, however with this method the ISTD cannot compensate for variations related to sample extraction.

Objective: To evaluate DBS analysis of drugs of abuse for its application in forensic toxicology, including determination of optimal methods for ISTD addition, and whether special considerations should be taken when analyzing DBS of postmortem blood.

Method: Three methods of ISTD addition were evaluated using human whole blood spiked with amphetamine (AMP), methamphetamine (MAMP), MDA, MDEA, and MDMA. Method A: adding ISTD to the blood prior to spotting, Method B: adding ISTD onto the blank card and allowing it to dry prior to applying the blood spot, and Method C: adding ISTD into the extraction solution. DBS spiked with benzoylecgonine (BZE) and cocaine (COC) were evaluated by ISTD and by Method D: adding ISTD into tubes containing the punched DBS. All samples were spotted onto Whatman 903 Protein Saver cards and allowed to dry at room temperature for at least 3 hours. Each DBS spot was punched (3 mm diameter) and placed into microcentrifuge tubes. AMP, MAMP, MDA, MDEA and MDMA were extracted using sodium hydroxide and ethyl acetate. The organic layer was placed into a test tube followed by methanolic hydrochloric acid, evaporated under nitrogen at 40^{o}C and reconstituted in mobile phase. BZE and COC were extracted using saturated sodium chloride and acetonitrile followed by evaporation and reconstitution of the organic layer. DBS of thirty-one postmortem blood samples previously confirmed for COC and BZE were extracted using method D described above. Whole blood samples were extracted following the same methods. All samples were analyzed by LC-MS/MS (Agilent 1290-6490).

Result: As expected**,** adding ISTD into blood prior to spotting resulted in the best accuracy and % CV (87-106 %, <4 %). The % CV for all AMP, MAMP, MDA, MDEA and MDMA was lower when ISTD was added into the extraction solvent (4.9 %) than when added to the card (6.6 %). For BZE and COC, the % CV for ISTD addition methods C and D were comparable. DBS prepared from postmortem samples quantified higher than when the same postmortem samples were extracted as whole blood (% difference=62) when the DBS calibration curve was prepared from antemortem blood. However, when postmortem blood was used to prepare the DBS calibration curve, the % difference decreased to 7. When ISTD was added directly to the blood, postmortem DBS samples could be quantified accurately using an antemortem calibration curve.

Conclusion: For antemortem blood, adding ISTD into the extraction solvent or into the microcentrifuge tube provided the most accurate and reproducible results. For postmortem blood, in order to obtain accurate results, ISTD must be added into the blood prior to spotting onto the card or postmortem blood must be used to prepare the calibration curve.

Keywords: Postmortem, Dried Blood Spots, Internal Standard

P97

Validation of an Automated Solution for Post Hydrolysis Enzyme Removal and LC/MS/MS Analysis of Illicit and Pain Related Compounds from Urine

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Background/Introduction: The use of illicit drugs and the escalating number of prescription drugs to treat chronic pain has mandated that health care providers routinely perform urine drug testing. Most laboratories adopt a "dilute-and-shoot" approach, using an enzymatic hydrolysis procedure to de-conjugate the glucuronidation metabolite back to the active form for many drugs. Addition of the enzyme (β-glucuronidase in this case) increases the protein concentration in the urine matrix. Insoluble in HPLC solvents, these proteins could precipitate throughout the chromatographic run, resulting in a flow path disruption and/or obstructed column inlet. The use of a simple procedure to remove the proteins from hydrolyzed urine samples prior to analysis will extend the LC column lifetime, as well as the assays robustness. Furthermore, the use of an automated fluid handling system to perform the protein removal procedure increases the laboratory productivity and reduces potential errors.

Objective: Incorporating fluid handling automation with protein precipitation, we present an automated solution to remove the β-glucuronidase from urine samples. This method was corroborated by validating in a CLIA regulated laboratory for the quantitation of 51 illicit and pain related drugs.

Method: Hydrolysis of 500µL of urine was performed by diluting with 100µL acetate buffer (pH 4.5-4.8) and 20µL of β-glucuronidase, 10^5 units/mL (DR2100, Campbell Scientific). The samples were vortexed for 10-15 seconds and then incubated at 63C for 30min. 100µL of hydrolyzed sample was loaded onto an Impact Protein Precipitation Plate, containing 400µL of methanol. The plate was sealed and vortexed for 2min, and a vacuum of 2-7" of Hg was applied for 2-3 minutes, collecting the filtrate. The filtrate was evaporated to dryness, and reconstituted in starting mobile phase before being transferred for LC/MS/MS analysis. The protein precipitation protocol was performed on a Tecan Freedom EVO 200 Fluid Handler. LC/MS/MS analysis was performed on an AB SCIEX QTRAP 6500, using ESI Positive ionization. Chromatographic separation of 51 compounds was achieved on a Phenomenex (Torrance, CA) Kinetex 2.6µm Phenyl Hexyl 50x4.6mm column. Mobile phase include a 0.1% formic acid and methanol gradient.

Result: A robust methodology for 51 illicit and pain related compounds was developed and validated. Over 500 injections were performed during validation, with no measurable increase in system backpressure. Furthermore, this methodology demonstrates good sensitivity, precision (Within run, Within laboratory, Inter and Intra-day reported) at low and high concentrations, as well as meets matrix effects requirements of ± 50%. This method was validated and currently used at Precision Toxicology Laboratories.

Conclusion/Discussion: The presence of additional protein (β-glucuronidase) in a urine sample can denature in the column during a normal chromatographic run. As a result the HPLC column will plug and potentially cause the run to stop due to over-pressurization of the system. The removal of this protein from the samples by a simple protein precipitation procedure can enhance the longevity of the column while not affecting the performance of the LC/MS/MS methodology.

Keywords: β-Glucuronidase, Dilute-and-Shoot, Pain Panel

P98
NBOMe — A Deadly LSD Look-A-Like

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Background/Introduction: NBOMe compounds are serotonin (5-HT_{2A}) receptor agonists that produce intense hallucinations. This psychedelic class of drugs is generally consumed from blotter paper like LSD and has emerged in the teenager/young adult scene. Many think the drug is harmless due to the common names like Happy and Smiles and packaging that include playful images like clowns. The concentration of these NBOMe compounds in postmortem cases is extremely low, usually below 0.50 ng/mL. A sensitive analytical method to quantify 6 NBOMe analogues was necessary due to a request from our medical examiners.

Objective: The objective of this poster is to discuss the validation of an LC/MS/MS NBOMe method and to present cases involving these drugs. The NBOMe series is a class of synthetic drugs, including 25b-NBOMe, 25c-NBOMe, 25d-NBOMe, 25h-NBOMe, 25iNBOMe, and 25T2-NBOMe. They are psychedelic compounds derived from the substituted phenethylamine psychedelic 2C-I (2,5-dimethoxy-4-iodophenethylamine) that are potent serotonin (5-HT_{2A}) receptor agonists.

Method: For this validation, and case analysis we adopted a solid phase extraction procedure that required 500 µL of sample. This validation included the most commonly analyzed matrices; whole blood, plasma, and urine, while utilizing a deuterated internal standard of similar structure to ensure extraction and chromatographic characteristics consistent with the analytes of interest. The samples were analyzed by ABSciex 4000 QTrap a liquid chromatography-tandem mass spectrometry (LC/MS/MS) instrument. The method utilizes Ultra Performance Liquid Chromatography (UPLC) technology for separation; followed by positive-electrospray ionization (ESI) of each analogue. Limits of quantitation (LOQ) for these analogues ranged from 0.01 to 0.02 ng/mL (10 to 20 pg/mL). SWGTOX method validations guidelines were reference during this validation.

Result: We had three known NBOMe-related fatalities at the Tarrant County Medical Examiner's Office, ranging from 19 – 22 years old. We found 25b-NBOMe and 25h-NBOMe in all three cases and in one of the cases; 25c-NBOMe was detected as well.

Conclusion: This is a reliable and sensitive method for the identification and subsequent quantitation of 6 NBOMe compounds in biological specimens. This procedure can be used on whole blood, plasma, and urine specimens both antemortem and postmortem cases and was recently published in the *Journal of Analytical Toxicology*.

Keywords: NBOMe, Validation, Fatalities

P99

Correlation of Blood Concentrations and CB1 Effects in Mice Following Inhalation of CP47,497 Smoke

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Introduction: CP47,497, a cyclohexylphenol ("CP") non-classical synthetic cannabinoid is one of the major 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 vitro studies. Initially synthesized in the 1980s as a pharmacological tool to help elucidate the structure of the cannabinoid receptor(s) in the 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. Reported toxicity symptoms 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'.

Objective: The objective of this study was to demonstrate the bioavailability of CP47,497 when smoked on dried plant material and determine if blood concentrations following smoke inhalation were dose dependent. Additionally, the data will be evaluated to determine if these CP47,497 blood concentrations correlate with quantifiable CB1 pharmacological effects.

Method: Correlation of CP47,497 CB1 effects in the mice were evaluated using the well-established Martin Tetrad behavioral model consisting of four outcome measures; catalepsy, body temperature, spontaneous locomotor activity and antinociception. Groups of six ICR mice were exposed to smoke from one of 3 doses; 5, 10 or 20mg of CP49,497 added to 200mg of placebo marijuana (THC free) obtained from the National Institute of Health. Spontaneous activity was captured 5-15 minutes after the inhalation; catalepsy, antinociception and temperature were assessed 30 minutes after. Blood was collected via cardiac puncture between 45-60 minutes after inhalation. Blood CP47,497 concentrations were analyzed using a validated HPLC/MS/MS method.

Result: One hour after inhalation of 5, 10 or 20mg CP47,497 doses, blood concentrations increased in a dose dependent manner, 12.85ng/ml (±3.89), 62.74 ng/ml (±19.09) and 98.3ng/ml (±18.46), respectively. No drug was detected in blood following inhalation of placebo marijuana. Catalepsy increased with increased doses of CP47,497 to 40.78 ± 9.3 at 20mg (p-value = 0.0011). Body temperature decreased with increased doses to -6.7 ± 0.5°C at 20mg ($p = 0.0007$). Spontaneous activity decreased significantly with increasing doses of CP47,497 to 87 ± 20 units at 20mg ($p = <0.0001$). Antinociception was less dramatic and only increased with the 5mg (13.89 ± 6.3) and 10mg (19.94 ± 9.1) doses of CP47,497, and returned to the 5 mg dose at 20mg (12.2 ± 6.6) (p-value >0.05). These CB1 effects were not observed in mice treated with placebo marijuana.

Conclusion: CP47,497 blood concentrations correlated with the 5, 10, or 20 mg doses of CP,497. Behavioral measures using the Martin Tetrad assay demonstrated that mice exposed to smoke containing CP47,497 exhibit cannabimimetic effects consistent with dose dependent CB1 receptor agonist activity.

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Keywords: CP47,497, Cannabimimetic, Synthetic Cannabinoid, Spice Inhalation

P100

Development and Validation of a New Homogeneous Immunoassay for the Broad Detection of Indazole Carboxamide Synthetic Cannabinoids: AB-PINACA and ADB-PINACA Analogues and their Metabolites in Urine

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Introduction/Objective: Cannabimimetic indazole carboxamide derivatives, AB-PINACA, 5F-AB-PINACA, AB-FUBINACA, ADB-PINACA, 5F-ADB-PINACA, and ADB-FUBINACA are new synthetic cannabinoids entering the market. Although law enforcement worldwide have attempted to regulate and prevent the inflow of the synthetic cannabinoids to their countries; new designer drugs have continue to circumvent the laws and current drug testing methods leading to new types of synthetic cannabinoids. The objective of this project was to develop and validate a new high throughput homogeneous enzyme immunoassay (HEIA) for the rapid and broad detection of the indazole carboxamides synthetic cannabinoid metabolites in human urine.

Method: An anti-AB-PINACA and ADB-PINACA polyclonal-based homogeneous immunoassay was developed and validated with LCMS confirmed urine specimens. The assay was designed to detect the indazole carboxamides metabolites in urine.

Result: The reportable range of the assay was 5 to 40 ng/mL while the cutoff concentration of AB-PINACA pentanoic acid was set at 10 ng/mL. The intra-day and inter-day coefficient of variation (% CV) for the qualitative assay was less than 1.0%. The HEIA was validated with a total of 56 urine samples previously analyzed by LCMS. The sensitivity, specificity and accuracy of the assay were found to be 100%, 100% and 100%, respectively.

		Confirmation (1ng/mL)	
		N	**P**
HEIA (10ng/mL)	**N**	40	0
	P	0	16

Conclusion: A high throughput homogeneous enzyme immunoassay has been developed for the broad detection of indazole carboxamide synthetic cannabinoid metabolites in human urine. When applied to authentic specimens the assay correlated well with LCMS results.

Keywords: Enzyme Immunoassay, Indazole Carboxamide Synethetic Cannabinoids, AB-PINACA

P101

Evaluation of Patients Prescribed Adderall® and Vyvanse® for the Presence of Methamphetamine

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Introduction: Adderall® and Vyvanse are commonly prescribed medications, which may require compliance monitoring. A study was conducted on 297 patient specimens with prescriptions for Adderall® and 67 patient specimens prescribed Vyvanse®. Interpretation of the results was based upon scientific literature, which suggested patients administered Adderall® with urine concentration ratios of methamphetamine/amphetamine ≥0.5%, with similar isomeric distribution, may indicate polydrug use. This study included the evaluation of mass spectrometry results, prescribed medications, other drugs detected in the urine, gender and age of the patients.

Objective: Evaluate if methamphetamine was detected in patients' urine that were prescribed Adderall® and/or Vyvanse®. Additionally, determine if the presence of methamphetamine was due to an analytical artifact or a pharmaceutical impurity

Method: Specimens were extracted by LLE using deuterated internal standards, evaporated to dryness, reconstituted, and analyzed via UPLC/MS/MS. Specimens with UPLC/MS/MS methamphetamine concentration >5ng/mL were extracted by LLE using racemic propylamphetamine (internal standard) and N-trifluoroacetyl-l-prolylchloride for chiral determination. The organic layer was evaporated to dryness, reconstituted and analyzed via GC-ToF.
UPLC/MS/MS acceptance criteria included retention time ±2.5% with ion ratios ±20% of calibrators and QC results ±20% of the target concentration. GC/TOF criteria included relative retention time ±0.02 minutes with ion ratios ±30% of QC, QC results ±2 standard deviations of the target ratio, and accurate mass ±5mDa.
To determine if the presence of methamphetamine was an analytical artifact, a urine specimen was spiked with amphetamine at 100µg/mL. To determine if the presence of methamphetamine was an impurity, a 30mg Adderall XR® capsule, 10mg generic Adderall® pill, and a 50mg Vyvanse® capsule were dissolved in methanol at a 1mg/mL concentration. The specimen and solutions were extracted as described.

Result: Out of the 297 specimens prescribed Adderall®, 127 contained methamphetamine concentrations between the LOD (0.5ng/mL) and LOQ with methamphetamine/amphetamine ratios <0.5%. 79 specimens had positive (>LOQ) methamphetamine results of which 68 had ratios <0.5% with similar isomeric distribution, and methamphetamine ranged from 5-163ng/mL. The remaining 11 specimens had ratios >0.5% of which nine specimens had dextro or levo-methamphetamine isomeric distribution >90% with methamphetamine ranging 9.8-77,351ng/mL. The remaining two specimens had dextromethamphetamine isomeric distribution ranging 61-65% with concentrations of 8-71,725ng/mL.

Of the 67 specimens prescribed Vyvanse®, 40 contained methamphetamine between the LOD and LOQ. 13 specimens had positive methamphetamine concentrations ≥LOQ that ranged from 5.4-34.4ng/mL. 11 specimens had ≥90% dextroamphetamine/dextromethamphetamine isomeric distributions and two specimens were QNS for isomer analysis. Additionally, all specimens contained a methamphetamine to amphetamine concentration ratio ≤0.09%.
Buprenorphine was prescribed to 125 and detected in 134 of the total patients. Males consisted of 59.5% of the total specimens. Ages ranged between 8-70 and 11-68 for males and females, respectively.
The urine spiked at 100 µg/mL produced a result <LOD for methamphetamine for both mass spectrometry methods. The Adderall XR® capsule and pill produced amphetamine/methamphetamine on LC/MS/MS as well as the expected isomeric distribution. Upon extraction of lisdexamphetamine, only dextroamphetamine was detected in both mass spectrometry methods.

Conclusion: This study supports the conclusions described in the scientific literature, which indicated that ratios <0.5%, with similar isomeric distribution should be interpreted carefully as polydrug use may not be indicated. In the study, methamphetamine ranged 5-77,351ng/mL. Only 11of the Adderall® specimens contained amphetamine/methamphetamine ratios >0.5%. Furthermore, the dextro to levo-methamphetamine isomeric distribution was ≥90% for 9 specimens, which may support polydrug use. The remaining two specimens had similar isomeric distribution, which may indicate poly drug use with racemic methamphetamine. All positive Vyvanse® specimens had similar isomeric distribution with ratios ≤0.5%. Additionally, methamphetamine detected in the pharmaceuticals support the theory that methamphetamine detected with similar isomeric distribution and ratios <0.5% was likely due to an impurity.

Keywords: Vyvanse®, Adderall®, Isomeric Distribution

P102
Drug Metabolic Patterns in Oral Fluid: Are Urine Biomarkers Transferable?

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Background: Oral fluid (OF) testing continues to be a growing and viable alternative means of monitoring patients prescribed medications with abuse potential, facilitating determination of prescription adherence, and reducing risks from other non-disclosed concomitant drug use. Parent drugs are expected to be dominant in oral fluid. However, the presence of a biomarker is desirable to further substantiate drug use. Exploration into OF drug testing could be considered as a progression from urine drug testing and the target compounds in the analytical methods are usually the same between matrices. Drug metabolic patterns in oral fluid are an important factor in result interpretation, but supporting literature is not yet as extensive as for urine (Cone and Heustis, 2007).

Objective: In a random cross-sectional study, the metabolic patterns of selected drugs in oral fluid from a medication monitoring population were assessed.

Method: Oral fluid specimens (n= 13,780) were collected from chronic pain patients, using the Immunalysis Quantisal™ sampling device, and confirmed for common drugs of abuse, opioids and benzodiazepines by liquid chromatography tandem mass spectrometry (LC/MS/MS) as part of a medication monitoring program. Analysis was completed on an ABSciex Triple Quadrupole 4500 platform with an Agilent 1290 chromatographic system using validated methods. The methods only monitored the traditional urinary compounds associated with these drugs and excluded hydrolysis or monitoring intact glucuronides. Limits of quantification (LOQs) ranged from 1-10 ng/mL. Positive results (>LOQ) were compiled and interrogated for metabolic trends. Only samples with associated medications listed were counted for the prescription drugs.

Result: This assessment is limited by the method LOQs, unknown time of last dose and the reference medication list provided. Overall, parent drug was almost always detected and the presence of metabolite alone was uncommon. For MDMA, MDA was present at significantly lower relative concentrations, consistent with metabolism, and incidence directly related to MDMA concentration. Heroin presented mostly as 6-monoacetylmorphine (6-AM) with non-prescription morphine (MOR) only, or as 6-AM with both non-prescription morphine and non-prescription codeine (COD), with morphine>codeine in all but two instances. The latter being consistent with codeine presence as a heroin impurity. Parent heroin was not monitored. Detection of both parent and respective metabolites, was consistent, for cocaine (benzoylecgonine) and buprenorphine (norbuprenorphine) with detection of metabolite alone particularly uncommon for buprenorphine. Oxycodone (OC) was found predominantly with noroxycodone (NOC) only, or by itself, while oxymorphone (OM) detection was infrequent. Hydrocodone (HC) was found predominantly by itself, or with norhydrocodone (NHC) only, while hydromorphone (HM) detection was rare. Like hydrocodone, fentanyl, methadone and codeine were present in oral fluid predominantly as parent drug. Norfentanyl and norbuprenorphine were typically present at lower concentrations than parent drug concentrations compared to other drug/metabolite combinations. Notably, for alprazolam, α-hydroxyalprazolam was not detected; only parent drug was present. As a contrast, diazepam (DIA) was rarely found identified by parent drug alone. Instead, diazepam use was largely identified as nordiazepam (NORDIA) only. Distinctively, oxazepam (OXA) was always absent; temazepam was not monitored. Clonazepam was never found alone, but predominantly as 7-aminoclonazepam only.

Conclusion: Oral fluid has its own unique metabolic pattern, but most urinary biomarkers are transferable, and may have better value to supplement corroborated drug use with assessment at picogram levels. Urinary metabolites were often detectable in OF, but were not as extensively present in tandem or in place of parent drug as in urine, except for clonazepam and diazepam. This should be considered when interpreting oral fluid data. Nevertheless, in all cases assessed, except alprazolam, the monitoring of the urinary metabolites in oral fluid provided added assurance of drug use.

Keywords: Oral Fluid, Biomarker, Urine

P103
Capturing True Positivity for Tricyclic Antidepressants in Urine

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Background: Tricyclic antidepressants (TCAs) are prescribed for treatment of depression and other mood disorders, in addition to chronic pain, and are targeted in therapeutic drug monitoring. Traditional TCA analysis involves non-hydrolytic urine testing for parent drugs, some of which are metabolites of each other. A review of LC/MS/MS confirmation results for patients prescribed common TCAs revealed a higher than expected rate of potential non-adherence; one that exceeded the rate noted with immunoassay screen. Less than 1% of any parent TCA is excreted in urine; rather they are present predominantly as glucuronides of the parent drug with unbound and/or glucuronidated hydroxy metabolites. TCAs form quaternary ammonium-linked glucuronides, which have proven difficult to hydrolyze with conventional beta-glucuronidases. Recent research has demonstrated improved detectability of amitriptyline and cyclobenzaprine via hydrolysis using a recombinant enzyme. The availability of reference standards for hydroxy metabolites is also no longer a limiting factor for accurate TCA monitoring.

Objective: The applicability of hydroxy metabolites as better urinary biomarkers for capturing true positivity for TCA use was investigated. The impact of sample hydrolysis on detectability for other TCAs was also assessed.

Method: 8-Hydroxyclomipramine, 10-hydroxydesipramine and 4-hydroxynortriptyine were incorporated into an existing LC/MS/MS method that monitored the TCAs, amitriptyline, nortriptyline, imipramine, desipramine, clomipramine, doxepin, and the tricyclic muscle relaxant, cyclobenzaprine. Randomly selected urine patient samples with an associated TCA prescription were sequestered and refrigerated until re-analysis for comparison (amitriptyline n=20, nortriptyline n=7, imipramine n=2, desipramine n=1, clomipramine n=4, doxepin n=12, and cyclobenzaprine n=21). Samples were treated 1:10 with a mastermix of IMCSzyme™ recombinant beta-glucuronidase enzyme (~1000units), buffer (pH 7.5), and matching deuterated internal standards. Hydrolysis occurred in the transfer time between aliquoting and injection on instrument, with no additional incubation time or heat. Analysis was completed without further sample preparation on a Waters Acquity UPLC® TQD using the modified previously validated "dilute-and-shoot" method.

Result: A sample was considered positive by the presence of parent drug and/or metabolite(s) above LC/MS/MS method limit of quantification, 100 ng/mL. 4-Hydroxynortriptyline was detected at as much as 12X the concentration of unbound nortriptyline. 8-Hydroxyclomipramine was detected at concentrations 4-11X that of parent clomipramine. 10-Hydroxydesipramine was detected at 3-105X the concentration of unbound desipramine. All samples positive for hydroxy metabolites were also positive for the corresponding parent compounds nortriptyline, clomipramine and desipramine in hydrolyzed and unhydrolyzed samples. Hydrolysis improved the positivity rate by 6-90% for the various TCAs, except for desipramine (all positive without hydrolysis) and nortriptyline, for the prescription data subset. Nortriptyline samples that remained negative post-hydrolysis are probably true negatives as these samples had screened negative as well. An additional set of patient samples with positive TCA screens, but no associated TCA prescription was analyzed for an expanded hydroxy metabolite evaluation. Hydrolysis increased the positivity rate in this non-prescription subset by 13-75% for cyclobenzaprine (n= 8), doxepin (n= 8) and amitriptyline (n=19). The other TCAs were either all positive without hydrolysis or not represented in this data subset. Hydrolysis was most impactful for cyclobenzaprine and doxepin detection. Hydrolyzed samples consistently registered higher concentrations for the parent TCAs and hydroxy metabolites by two-fold or more compared to the original non-hydrolysis confirmation data, except for desipramine and nortriptyline, which do not form *N*-glucuronides.

Conclusion: Hydrolysis with a recombinant beta-glucuronidase increases the positivity rate of tricyclic antidepressants with tertiary amines. Even without sample hydrolysis, hydroxy metabolites had no substantial impact on positivity rate, since they were detected in tandem with the associated parent compound. However, they do substantiate drug use for their corresponding parent drug.

Keywords: TCAs, Urine, Positivity

P104

Development of a Highly Sensitive and Specific ELISA for AB-PINACA, ADB-PINACA, Analogous Indazole and Indole Carboxamides and their Metabolites in Urine

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Introduction: While previous generations of synthetic cannabinoids, JWH-018, UR-144 and PB-22 are currently part of toxicology laboratory drug screens, the newest synthetic cannabimimetics to emerge over the last 24 months have been the PINACA compounds. These compounds consist of a core indazole carboxamide structure, with a pentyl side chain similar to JWH-018, or a modification thereof. Currently, AB-PINACA, ADB-PINACA, AB-FUBINACA and AB-CHMINACA are DEA schedule I substances. Due to the reported health risks involved with their use, as well as them being controlled substances, it becomes imperative to provide toxicologists with a reliable and sensitive screening method to test for the presence of the PINACA compounds and their metabolites in urine.

Objective: To develop a highly sensitive ELISA for the detection of AB-PINACA, ADB-PINACA, 5F-AB-PINACA, 5F-ADB-PINACA, AB-FUBINACA, ADB-FUBINACA, AB-CHMINACA, related analogues and their main metabolites in human urine.

Method: A polyclonal antibody was developed, having a broad cross-reactivity to PINACA compounds and further immobilized on a microtiter plate. ADB-PINACA N-pentanoic acid calibrators (10µL) and urine specimens (10µL) were pipetted in duplicate on the microtiter plate, immediately followed by addition of PINACA-HRP enzyme conjugate (100µL) and incubated for 60 min. The plate was washed with DI water, then incubated with TMB substrate (30 min), then stopped with 1N HCl and read at 450 and 650 nm, using a Tecan microplate reader.

Result: The ELISA utilizes ADB-PINACA N-pentanoic acid calibrator, to achieve a 2 ng/mL cutoff. The assay shows a high cross-reactivity (80-160%) to the N-pentanoic acid, N-(4-hydroxyl) and N-(5-hydroxyl) metabolites of AB-PINACA, ADB-PINACA, 5F-AB-PINACA and 5F-ADB-PINACA. It also cross-reacts with AB-CHMINACA, AB-FUBINACA and ADB-FUBINACA (40%), as well as shows 20-40% cross-reactivity to ADBICA, 5F-ADBICA, 5F-ABICA and their urinary metabolites. The assay was validated with 68 urine specimens obtained from clinical laboratories. 24 were true positives and 44 were true negatives, correlating with LC-MS/MS data. The assay showed intra and inter-day imprecision CV<10%, with a detection limit (LOD) of 0.5 ng/mL.

Conclusion: We have developed a new ELISA to meet the ever-changing need of screening for the latest synthetic cannabinoids in urine, i.e. the PINACA compounds and their metabolites. The assay is highly sensitive, with a cutoff concentration of 2 ng/mL and LOD of 0.5 ng/mL.

Keywords: AB-PINACA, ADB-PINACA, ELISA

P105

Extraction of Δ^9-THC, 11-hydroxy-Δ^9-THC and 11-nor-9-carboxy-THC from Whole Blood and 11-nor-9-carboxy-THC from Urine Using Supported Liquid Extraction (SLE) Prior to GC/MS Analysis

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Introduction: Cannabis misuse continues widely all over the world, and this has led to the necessity for rapid and reliable methods for the analysis and quantitation of THC and its metabolites in various matrices.

Objective: The objectives were to develop GC/MS assays for the determination of THC, THC-OH and THC-COOH from whole blood and THC-COOH from urine using supported liquid extraction (SLE).

Method: Blank whole blood was spiked with THC, THC-OH and THC-COOH and THC-D3, THC-OH-D3 and THC-COOH-D3 were used as the internal standards. Extraction conditions were evaluated using spiked whole blood pre-treated 1:1 (v/v) with 0.1% formic acid or 50 mM ammonium acetate to give a total of 750 µL on ISOLUTE SLE+ 1mL capacity columns. The BeadRuptor24 was evaluated prior to sample application to determine any binding disruption effects. Increased loading volumes and increased percentages of whole blood were evaluated to improve LLOQs.

Blank urine was spiked with THC-COOH and THC-COOH-D3 was used as the internal standard. Extraction conditions were evaluated using pre-treatments of 0.1% and 1% formic acid or HPLC-grade water 1:1 (v/v) on ISOLUTE SLE+ 1 mL capacity columns. Various urine-hydrolysis approaches were investigated. Beta-glucuronidase enzyme from Helix pomatia (50 µL/mL of urine) was used within a pH environment of ~5.5, achieved with ammonium acetate. Also evaluated was 10N potassium hydroxide (KOH) (100 µL/mL of urine). Both hydrolysis approaches were performed at temperature; enzyme for 2 hours at 37 °C and KOH for 25 minutes at 60 °C. Post hydrolysis, the KOH approach required the addition of glacial acetic acid to lower the pH environment to ~5.5.

Analyte extraction for both matrices was evaluated using MTBE, DCM, hexane and EtOAc. Both whole blood and urine extracts were evaporated with air at 40 °C. Derivatization was performed using 40 µL EtOAc and 20 µL BSTFA. The samples were vortex mixed and transferred to glass vials with non-split caps prior to heating at 70 °C on a heat block for 25 minutes. 1 µL was then used for GC/MS analysis in splitless 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: Whole blood results using the BeadRuptor24, loading 600 µL whole blood and 200 µL 0.1% formic acid (aq) for 800 µL in total, gave recoveries >74%, with RSDs <9%. LLOQs were 1 ng/mL for THC and 3 ng/mL for the metabolites. Hydrolyzed and non-hydrolyzed urine extractions gave THC-COOH recoveries >78%, with RSDs <10%. LLOQs were between 6-10 ng/mL for unhydrolyzed urine, 10-15 ng/mL for KOH hydrolysis and 15-30 ng/mL for enzyme hydrolysis. Linearity experiments demonstrated r^2 >0.994 for whole blood and >0.997 for all urine protocols, with concentrations 1-150 ng/mL.

Conclusion: This poster describes quick, reliable protocols for the extraction of Δ^9-THC, and its metabolites from whole blood and for the carboxy-metabolite from urine prior to GC/MS, demonstrating high, reproducible extraction efficiencies.

Keyword: SLE+ (Supported Liquid Extraction), Whole Blood, Urine

P106

Evaluation of Drugs of Abuse Extraction from Whole Blood Using Supported Liquid Extraction (SLE) Prior to GC/MS Analysis

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Introduction: Whole blood continues to be a valuable tool in forensic toxicology for the immediate and near-term detection of illicit drugs, and in cases where no other sample is available. 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 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 suites from a whole blood sample using supported liquid extraction (SLE) prior to GC/MS analysis and achieve recovery percentage of greater than 70%. The drug suites were amphetamines, barbiturates, benzodiazepines, cocaine, and opiates.

Method: Blank whole blood was spiked with drugs from the five drug groups stated in the objectives. Amphetamine-D5, Diazepam-D5, Benzoylecgonine-D3, Morphine-D3 and 6-MAM-D3 were used as the internal standards. Extraction conditions were initially evaluated using spiked whole blood pre-treated 1:1 (v/v) with 50 mM ammonium acetate ~ pH 8 or 1% ammonium hydroxide (aq) to give a total of 750 µL on ISOLUTE SLE+ 1 mL capacity columns. Increased loading volumes and increased whole blood volume in the load was evaluated with the aim to improve LLOQs while maintaining cleanliness. The BeadRuptor24 was evaluated alongside traditional vortex-mixing prior to sample loading, to determine any binding disruption effects.

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. Following extraction, if amphetamine analysis was the end-point, 100 µL 0.2M HCl in methanol was added to the solvents for the purpose of decreasing the volatile character of amphetamine by forcing a salt conversion. Evaporation was performed with air at ambient room temperature.

Derivatization was performed depending on the desired drug group. For amphetamines, 30 µL EtOAc and 30 µL heptaflurobutyric anhydride (HFBA) was added, vortex-mixed and transferred to glass vials prior to heating at 70 °C on a heat block for 25 minutes. Following this, the samples were evaporated at ambient room temperature to dryness and reconstituted with 60 µL EtOAc.

For benzodiazepines, cocaine and opiate analytes, 30 µL EtOAc and 30 µL BSTFA was added, vortex mixed and transferred to glass vials prior to heating at 70 °C on a heat block for 25 minutes.

For barbiturates, 80 µL ethyl acetate and 20 µL TMAH (trimethylanilinium hydroxide, 0.2M) was added, vortex mixed and transferred to glass vials.

2 µL was then used for GC/MS analysis in split-less 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: Upon loading 500 µL whole blood and 250 µL 1% ammonium hydroxide(aq) for 750 µL in total, recoveries were greater than 70% with RSDs below 10%.
LLOQs were 5-20 ng/mL for amphetamines, 2-10 ng/mL for barbiturates, 10-20 ng/mL for benzodiazepines, 10 ng/mL for cocaine analytes, and 10-20 ng/mL for opiates.

Linearity experiments demonstrated r^2 >0.99 for all analytes, with concentrations 5-500 ng/mL.

Conclusion: This poster describes reliable, fast protocols for the extraction of multiple drug groups and metabolites from whole blood prior to GC/MS, demonstrating high, reproducible extraction efficiencies.

Keywords: SLE+ (Supported Liquid Extraction), Whole Blood, Drugs of Abuse, GC/MS

P107
Opiates in Blood by LCMS: A Simple Derivative to Improve the Chromatography of Keto-Opiates

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Background/Introduction: The analysis of blood for opiates using UPLC-MSMS is sensitive and fast (6 minute run time) with sharp chromatographic peaks (fwhm≈0.1min). However, the keto-opiates (oxycodone, oxymorphone, hydrocodone, and hydromorphone) exhibit unusual chromatography, displaying a slow baseline buildup before the chromatographic peak. This peak-fronting is observed on all the keto-opiates but is most prominent for oxycodone. The problem is that the fronting appears as a sloping baseline and causes inconsistent integration of the peak.

Objective: This article presents two similar derivatizing agents that are simple to use and improve the peak shape of the keto-opiates (hydroxylamine and methoxyamine). Two different chromatographic columns were investigated for the best chromatographic separation of each derivative

Method: Solid phase extraction, UHPLC-MSMS with ESI

Samples preparation: 0.5 mL of whole blood, 3mL of pH9 carbonate buffer (0.3M) and 25uL of internal standard mix (resulting in 100ng/mL) were added to a test-tube. The test-tube was thoroughly vortexed and centrifuged at 4400 g for 10 minutes. The liquid portion was poured into a SPEware-*Clin-II* solid-phase extraction (spe) column. The spe-column was washed with 1mL HCL (0.1M), followed by 1mL methanol and finally 1mL ethyl acetate(EA). The analytes were eluted with 1.25 mL EA/n-propanol/NH_4OH (90:10:5) directly into a 2mL-autosampler vial. The eluate was dried under a stream of nitrogen at 50°C. 100 uL of derivatizing reagent was added (either 5% methoxyamine or 5% hydroxylamine in water containing 50% methanol). The autosampler vials were allowed to sit 10 minutes at room temperature for the completion of the oxime reaction before additional 250 uL water containing 0.1% formic acid was added.

Instrumental: The detector was an Agilent 6460 triple-quadrupole with ESI in the positive-ion mode. The liquid chromatograph was an Agilent 1200 (max. pressure: 600 bar) with a 0.2µm in-line frit to protect the analytical column. The injection volume was 2-10 µL. The column temperature was 30° C. For each derivative (methoxyamine and hydroxylamine) two different analytical columns were evaluated: a) Zorbax Eclipse-Plus C18, 1.8um, 2.1x50mm and b) Raptor biphenyl 2.7um, 2.1x50mm. The liquid chromatograph parameters varied with column and derivative, the "A" mobile phase was water with 0.1% formic acid, the "B" mobile phase was either MeOH or acetonitrile with 0.1% formic acid.

Result: Derivatization with either hydroxylamine or methoxyamine results in two chromatographic peaks for each keto-opiate, presumably due to the syn and anti-configuration of the derivative with respect to the nitrogen double-bond (Meatherall, Broussard). Depending on the choice of chromatographic column, the resulting peaks can be well separated with a clean baseline or they can completely coalesce into a single peak for each keto opiate. The assay was validated with a calibration range of 2-250 ng/mL for all seven opiates (codeine, morphine 6MAM, hydrocodone, hydromorphone, oxycodone and oxymorphone).

Conclusion/Discussion: The oxime-derivatization of the keto-opiates using either hydroxylamine or methoxyamine improves their chromatography. The derivatization-reaction is easy to incorporate into the preparation of the sample (either pre- or post-extraction) so that little or no extra sample-prep time is needed.

Keywords: Keto-Opiates, LCMS, Derivative

P108

Concentrations of Morphine and Codeine in Paired Oral Fluid and Urine Specimens Following Ingestion of a Poppy Seed Roll and Raw Poppy Seeds

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Introduction: Interpretation of opiate drug test results can be challenging due to casual dietary consumption of poppy seeds which may contain variable opiate content.

Objective: The purpose of the present study is to investigate opiate analytical results in paired urine and oral fluid and specimens, following ingestion of poppy seeds of known opiate content, using the Oral-Eze® Oral Fluid Collection System and the CEDIA® Opiate OFT Assay, an FDA-cleared collection and testing system, with confirmation by Gas Chromatography- Mass Spectrometry (GC-MS).

Method: Opiate concentrations in paired oral fluid (OF), collected with the Oral-Eze Oral Fluid Collection System, and urine were analyzed after ingestion of poppy seeds from the same source, consumed raw or contained in a roll. In Part-1, 12 individuals consumed equal portions of a poppy-seed roll. For Part-2, the same individuals consumed an equivalent quantity of raw poppy seeds, containing approximately 3.2 mg morphine and 0.6 mg codeine. Specimens were analyzed both by enzyme immunoassay (opiates) and GC-MS (morphine/codeine).

Result: Urinary morphine was between 155-1408 ng/mL (roll) and 294-4213 ng/mL (raw), measured at 2, 4, 6 and 20 hours post-ingestion. Urinary codeine concentrations between 140-194 ng/mL (roll) and 121-664 ng/mL (raw) were observed up to 6 hours post-ingestion. Following raw poppy seeds, OF specimens were positive, above LOQ, from 0.25-3.0 hours with morphine ranging from 7-600 ng/mL and codeine from 8-112 ng/mL. After poppy seed roll consumption, morphine concentrations of 7-143 ng/mL were observed up to 1.5 hours with codeine detected in only 5.5% of OF specimens and ranging from 8-28 ng/mL.

Conclusion: Combined with existing poppy seed literature, these results support previous findings and provide guidance for interpretation of OF opiate testing.

Keywords: Poppy Seed, Oral Fluid, Oral-Eze

P109

Development of a Hydroxyl-Position Determining Method for the Metabolites of Naphthoylindole-Type Synthetic Cannabinoids by GC/MS/MS

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Background/Introduction: One of the many issues of new designer drugs of abuse like synthetic cannabinoids (SC) is that they are extensively metabolized prior to excretion and multiple metabolites exist. Hydroxylated metabolites of the synthetic cannabinoid JWH-018 have been reported to retain in vivo and in vitro CB1 affinity and activity, thus characterization of the hydroxylated metabolites is of importance in forensic toxicology. The parent compound has many sites available for hydroxylation and thus site-dependent differences may exist in whether hydroxylation occurs depending on the metabolic pathways of such SCs. The existence of different hydroxylated metabolite isomers exemplifies the necessity for characterizing the specific metabolic pathways responsible for the biotransformation of the continuously emerging designer drugs. Determination of the location of these hydroxylation sites and differentiation of such isomers of the SC metabolites may aid in the further characterization of the metabolic pathways of SCs.

Objective: The aim of this study was to investigate in the hydroxyl-position determining method of metabolites of naphthoylindole-based synthetic cannabinoids by GC/MS/MS.

Method: Standard compounds of the SC hydroxyindole metabolite isomers were purchased from Cayman Chemicals. Underivatized and trimethylsilyl (TMS)-derivatized hydroxyindole metabolites were analyzed by GC-MS first in the scan mode to investigate those isomers who could be differentiated by EI scan spectra. Isomers with identical or near-identical EI spectra were further subjected to GC-MS/MS analysis with appropriate precursor ions. For the animal study, JWH-018 or MAM2201 were intraperitoneally administered to 6-week Wistar rats at 5 mg/kg dose (n=4 for each drug). Urine was pooled over 24 hours following administration. Urine samples were enzymatically hydrolyzed with beta-glucuronidase before protein extraction by methanol, followed by trimethylsilylation of the extracted residue. All instrumental analyses were performed on a Shimadzu GCMS-TQ8040.

Result: Among the four hydroxyindole metabolite isomers of JWH-018, EI scan was able to distinguish only one of the isomers, namely, the 4-hydroxyindole metabolite, showing a characteristic ion at *m/z* 414. The remaining 5-hydroxy, 6-hydroxy, and 7-hydroxyindole metabolites exhibited near-identical spectra with the TMS-derivatized molecular ion as the base peak, hence EI-MS/MS was performed by selecting *m/z* 302 (the indole moiety) as the precursor ion. The four isomers each demonstrated characteristic MS/MS spectra. The 4-hydroxy, 6-hydroxy, and the 7-hydroxyindole metabolites each produced characteristic product ions at *m/z* 174, *m/z* 204, and *m/z* 216, respectively, enabling differentiation between them. The 5-hydroxyindole metabolite in turn lacked these characteristic ions, consequently allowing for the differentiation among the other isomers. Results obtained for the urine samples from rats administered with JWH-018 and MAM2201 will also be discussed in the presentation.

Conclusion: EI-MS/MS allowed for the regioisomeric differentiation of the hydroxyindole metabolite isomers of the SCs investigated in this study. Results obtained from this study can be used as a hydroxyl-position determining method for the metabolites of naphthoylindole-type SCs by GC/MS/MS, which can be applied in characterizing the metabolic pathways of SCs.

Keywords: GC/MS/MS, Isomer Differentiation, Synthetic Cannabinoid Metabolites

P110

A Novel Screening Approach for the Detection of Toxicologically Relevant Substances

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Introduction: Laboratories are frequently required to perform broad screening techniques to identify toxicologically-relevant analytes in complex biological samples. A number of analytical techniques are available for comprehensive screening but more recently, time-of-flight (Tof) mass spectrometry has gained popularity due to the ability to perform both targeted and non-targeted analysis. In addition to mass accuracy, substances can be identified using supporting information such as retention time (RT) and fragment ions generated by MS^E (Rosano *et al.*, 2013). The combination of such measurements can reduce false positive assignments in screening assays. However for some substances, identification can remain challenging due to the presence of co-extracted matrix interferences or structural isomers. The incorporation of collision cross-section (CCS) data, generated through ion mobility separation (IMS) techniques, has been demonstrated to improve the accuracy of screening for pesticides in food (McCullagh *et al.,* 2014*)*. Thus the potential value of CCS data for toxicological analysis is of interest.

Objective: To evaluate the feasibility and potential benefits of CCS data for systematic toxicological analysis.

Method: Solvent standards for more than 50 molecules including opiates, amphetamines and benzodiazepines were analysed using UPLC interfaced with a novel IMS QTof mass spectrometer (Waters).
An established 15 min chromatographic method was used and the IMS QTof was operated in high-definition MS^E ($HDMS^E$) mode. This involves sequential ion mobility separation, where molecules can be distinguished on the basis of their 3D conformation, followed by MS^E analysis. MS^E comprises a rapid alternation between two collision energy functions: the low energy provides the accurate mass of the drift time-separated precursor ions whereas the elevated energy function provides the accurate mass of the fragment ions, for additional confirmatory purposes. IMS led to simplified elevated energy spectra during $HDMS^E$ analysis due to drift time alignment of fragment ions with their respective precursors. Reference CCS values were measured and subsequently added into an existing library (UNIFI Toxicology Screening Library; Waters) containing more than 1300 substances.

Preliminary Data: To assess the validity and reproducibility of the reference CCS values, blank urine and plasma samples enriched with the same drug standards, were analysed using UPLC-$HDMS^E$. In addition, a series of authentic biological samples were analysed. Data were processed and screened against the updated toxicology library. For all molecules investigated, observed CCS values were within 2% of reference values. The feasibility of using CCS as a potential substitute for RT was demonstrated by the successful application of an alternative chromatographic method with a shorter analytical run time.

Conclusion: Collision cross-section values were shown to be highly reproducible both in the presence or absence of complex biological specimens. IMS led to simplified low and elevated energy spectra during MS^E analysis due to the alignment of ions in drift time. CCS values provide a valuable additional dimension of specificity which proves valuable in systematic toxicological analysis.

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M.McCullagh, V. Hanot, and S. Goscinny, Use of Ion Mobility Spectral Cleanup and Collision Cross Section Values to Increase Confidence and Efficiency in Pesticide Residues Screening Strategies. Waters Application Note Library-720005080EN.

Keywords: Ion Mobility, Tof-MS^E, Toxicology Screening

P111

The Detection of Grayanotoxins in the Blood, Urine and Mad Honey from the Mad Honey Poisoning Patients

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Introduction: Grayanotoxins (GTXs) are toxic compounds contained in the leaves, twigs, flower, pollen and nectar of some species of the *Ericaceae* (*Rhododendron*) family. Honey produced from the floral nectar of *Ericaceae* (*Rhododendron*) family, which is known as 'mad honey', occasionally contains GTXs and causes poisoning. The GTXs are neurotoxins interfering with the transmission of the action potential by blocking sodium channels in cell membranes. GTXs poisoning symptoms are dose-related. In mild form, dizziness, weakness, excessive perspiration, hypersalivation, nausea, vomiting and paresthesia are present. GTXs may cause cardiac disturbances such as low blood pressure or shock, bradyarrhythmia, sinus bradycardia or nodal rhythm. Severe poisoning may lead to life threatening cardiac complcations such as complete atrioventricular block.

Method: A sensitive and specific method for the quantitation of Grayanotoxin I (GTX I) and Grayanotoxin III (GTX III) in the blood, urine and honey based on high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed and validated. Clindamycin was used as the internal standard (IS). After solid-phase extraction using PEP solid-phase extraction cartridges, Grayanotoxins (GTXs) were separated on a Kinetex Biphenyl (100 × 2.1 mm, 2.6 μm) column using a gradient elution with the mobile phase of 1 % acetic acid in water and methanol at a flow rate of 0.25 mL/min. Electrospray ionization (ESI) mass spectrometry was operated in the positive ion mode with multiple reaction monitoring (MRM).

Result: The assay of GTX I was linear from 5 to 1000 ng/ml in the urine and blood, from 0.02 to 20 μg/g in the honey. The assay of GTX III was linear from 20 to 5000 ng/ml in the urine and blood, from 1 to 1000 μg/g in the honey. The relative standard deviation of intra-day and inter-day precision was below 10.6 % and accuracy ranged from 94.3 to 114.0 %. The recovery was above 83.4 % for the analytes at three QC samples (low, medium, high). The validated method was successfully applied to the quantification of GTXs in the blood, urine and mad honey samples collected from 30 mad honey poisoning patients that came to the hospital. The samples analyzed were found to contain GTX I and GTX III ranging from 0.0 to 25.12 ng/ml and from 0.0 to 37.52 ng/ml in the blood, ranging from 0.0 to 0.145 μg/ml and from 0.0 to 1.90 μg/ml in urine, and ranging from 0.09 to 21.32 μg/g and from 0.0 to 74.11 μg/g in the mad honey.

Conclusion: There are no papers published for the determination and the quantification of GTXs in human blood and urine samples due to the lack of analytical sensitivity and low selectivity. This research is the first report of detection of GTXs in the blood and urine of patients suspected mad honey poisoning. These methods would provide a useful tool for reporting dangerousness of intake of mad honey and prevention of mad honey poisoning. This assay would help for doing the clinical and forensic studies of mad honey poisoning.

Keywords: Mad Honey, Grayanotoxin, LC-MS/MS

P112

Quantitation of Benzodiazepines and Z-Drugs in Serum with the Evoq LC Triple Quadrupole Mass Spectrometer

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Introduction: Benzodiazepines and the so called z-drugs are among the most subscribed drugs as they have sedative, hypnotic, anxiolytic, anticonvulsant and muscle relaxant properties but they also bear the risk of dependence. Traditionally they have been analyzed with gas chromatography or liquid chromatography (LC) with UV detector. Nowadays LC coupled to a triple quadrupole mass spectrometer is more commonly used as this technique has a better selectivity and sensitivity.

Objective: This study presents a method to simultaneously quantitate 18 benzodiazepines, metabolites and z-drugs in serum using liquid liquid extraction (LLE). A round robin test for 13 of the analytes organized by the German "Society of Toxicological and Forensic Chemistry" (GTFCh) was attended to proof the reliability of the method.

Method: Serum samples were extracted with LLE using chloroform/isopropanol (95:5). After separation and evaporation of the organic layer the residue was reconstituted in the UHPLC mobile phase without preconcentration. 1 µL extract were separated by UHPLC in only 11 minutes run time and analyzed by the Evoq triple quadrupole mass spectrometer with positive electrospray ionization in MRM mode.

Result: Quantitation was performed using deuterated internal standards in a concentration range of 0.5 – 500 ng/mL for most analytes. The calibration curves show good linearity with R^2 0.994 – 0.998. The limits of detection ranged from 0.05 to 2.5 ng/mL. The round robin test was passed for all 13 analytes that were included in the test with excellent z-scores between 0.0 and 1.3.

Conclusion: A quick and very sensitive LC-MS/MS method with LLE for the quantitation of benzodiazepines in serum is presented. Its suitability for routine analysis was proven by passing a round robin test.

Keywords: Benzodiazepines, Serum, LC-MS/MS

P113
A New Software Tool for Confident MRM Based Quantitation in Forensic Toxicology

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Introduction: In forensic toxicology confidence in the accuracy of quantitative results is of great importance. Currently, sample preparation and sample analysis can be automated, however data review and verification of chromatograms is usually a manual, time-consuming and tedious process that is prone to human bias or error. Typically in quantitative analysis when reviewing data quality, the review criteria and associated quality flags are based on the final calculated concentration. For results with calculated values outside of a permitted range, the quality flag visually indicates to the analyst the possible need for manual peak review and re-integration. However this 'concentration-based' evaluation can be too late in the process as results may be derived from poor chromatographic peak integration, leading to the acceptance of an incorrect quantitative result.

Objective: This poster will present a new quantitative software solution, based on the "downstream" chromatographic peak shape fidelity to significantly increase the reliability of quantitation for triple quadrupole data derived from both GC and LC based systems.

Method: We describe a new 'exception based review' quantitation software that initiates data review starting with the chromatographic peak integration itself. The software processes the data and flags chromatograms that do not fulfill the pre-determined chromatographic peak quality criteria allowing the analysts to efficiently utilize their time reviewing only those chromatograms. Integral to the solution is an exponentially modified Gaussian peak detection algorithm (EMG) that is able to identify minor peaks buried in the chemical or electronic noise. Any peaks that do not fulfill the peak integration criteria are flagged for immediate data review, regardless of the calculated concentration.

Result: With the new quantitation software the user saves time by only reviewing those chromatograms that do not comply with the pre-determined rules. Furthermore peak integration becomes more reproducible and manual integration to fulfil regulations is avoided.

Conclusion: The new exception based data-review software reduces the error rate for quantitative analysis whilst also improving laboratory throughput. Utilizing the EMG algorithm delivers reproducible, high fidelity peak integration, reducing the frequency of manual peak inspection and re-integration that was previously needed.

Keywords: Quantitation Software, Triple Quadrupole, Exception Based Reviewing

P114

A Novel Extraction for Buprenorphine, Norbuprenorphine, and Their Glucuronide Metabolites to Improve Detection for Butrans® Prescriptions

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Introduction: Buprenorphine is an opioid medication commonly prescribed to manage moderate to chronic pain or to treat opioid addiction. A transdermal patch formulation of buprenorphine (Butrans®) is indicated to manage chronic "around-the-clock" pain. Butrans®, unlike sublingual administrations of buprenorphine, presents lower concentrations of buprenorphine metabolites in urine warranting the need for lower detection limits for buprenorphine compliance monitoring.

Objective: This method was developed to detect buprenorphine, buprenorphine glucuronide, norbuprenorphine, and norbuprenorphine glucuronide at 2 ng/mL or lower in order to improve detection for patients with low buprenorphine dosages, in particular, patients prescribed Butrans®. Monitoring the glucuronide metabolites allows for better patient compliance assessment and identification of potentially adulterated samples; therefore hydrolysis was not currently desired or utilized in this method.

Method: Aliquots of drug-free urine were fortified with buprenorphine, buprenorphine glucuronide, norbuprenorphine, and norbuprenorphine glucuronide at concentrations of 0.5, 1, 2, 5, 10, 25, 50, 100, 250, 500, 750, 1,000, 1,500, and 2,500 ng/mL. Randomly selected authentic urine samples previously tested for buprenorphine, using a validated dilute-and-shoot method, were collected and stored at 4°C until analysis. A 500 µL volume of each sample was diluted 2x with 100 m*M* formic acid internal standard solution containing 0.5 µg/mL of buprenorphine-D4 and norbuprenorphine-D3 and 0.1 µg/mL of norbuprenorphine glucuronide-D3. Extraction of samples used CEREX® Trace-B 35mg/3mL columns and an ALD-III system from SPEware Corporation. Analysis was completed on a Waters Acquity® TQD System with a Waters Acquity UPLC® HSS SB, 2.1x50mm, 1.8 µm column. The column temperature was 50°C and the cycle time for the method was 2 minutes. Data was acquired in positive electrospray ionization mode with two selected ions for all analytes and internal standards and processed on Waters TargetLynx software.

Result: This method was validated following CAP guidelines for detection limits & linearity, carryover, precision & accuracy, extraction recovery, matrix effect, process efficiency, and patient correlation. The lower limits of quantitation were determined to be 2 ng/mL for buprenorphine, buprenorphine glucuronide, and norbuprenorphine, and 5 ng/mL for norbuprenorphine glucuronide. The upper limits of linearity and carryover were determined to be 2,500 ng/mL for all four analytes. All linearity points passed within 13.7% bias and with coefficient of variation less than 10.9%. Interday precision & bias was within 5.4% and 12.9%, respectively, while intraday precision & bias was within 4.5% and 11.8%, respectively. Extraction recovery was >75% for all analytes except norbuprenorphine glucuronide which was 25%. The matrix effects for all analytes were less than -25%, compared to the original dilute-and-shoot method where the analytes were suppressed between -44 and -81%. The overall process efficiency was >70% for all analytes except norbuprenorphine glucuronide which had an overall process efficiency of 20%. When comparing patient sample results between this extraction method and the original dilute-and-shoot method there was generally good agreement (within 30%); however, the general trend indicated that there was a positive % deviation bias for the extracted sample. This positive bias is likely due to the drastic decrease in matrix effect. An ~52% increase in positivity detection for patients prescribed Butrans® as a result of the extraction and the lower cut-off limits was observed.

Conclusion: Overall, this method was able to reduce the matrix effect in samples, enabling the achievement of lower reporting limits for buprenorphine and its major metabolites. The novel sample preparation protocol supports simultaneous extraction of glucuronide-bound and unbound analytes. This also allowed an improvement in capturing true positivity for buprenorphine in patients prescribed Butrans® by ~52%.

Keywords: Buprenorphine, Urine Testing, Butrans®, Extraction

P115

Evaluation of Clean Screen XCEL® II Extraction Columns in the Analysis of Delta-9-THC-COOH in Urine

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Introduction: The detection of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (delta-9-THC-COOH or THC-COOH) in urine has been used as a proof of the use of cannabis. In the laboratory, the current method of testing of THC-COOH in urine involves liquid-liquid extraction of a 5-ml sample which is time-consuming and laborious. Hence, an automated solid phase extraction method for THC-COOH in urine is evaluated using the Clean Screen XCEL® II extraction columns performed in an automated solid phase extraction system (Gilson GX-274 ASPEC). This will improve the sample analysis throughput as well as reducing the sample size from 5 to 2 ml urine.

Objective: The objective of this study is to develop and validate an automated solid phase extraction method for THC-COOH in urine using Clean Screen XCEL® II extraction columns.

Method: Two-ml urine samples were hydrolyzed at 60 ºC for 15 min after the addition of D_3-THC-COOH as internal standard and 50 µl 10M KOH solution. The samples were cooled and adjusted to pH 7 by the addition of 200 µl of 0.1M phosphate buffer (pH 7) and acetic acid/water (1:1 v/v) to each sample. The samples were then transferred onto the Clean Screen XCEL® II extraction columns and extracted using Gilson GX-274 ASPEC. After the sample loading, the columns were dried under positive pressure using nitrogen gas for 2 min. Subsequently, the columns were washed with 2 ml of hexane and dried for another 10 min by nitrogen gas. The samples were eluted with 2 ml of ethyl acetate/hexane/acetic acid (49:49:2 v/v). Derivatisation of the dried sample extract was performed using 50 µl of N,O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA) with 1 % Trimethylchlorosilane (TMCS) at 70 ºC for 15 min. The derivatised samples were reconstituted with 50 µl ethyl acetate before they were analysed by GC/MS using selected ion monitoring (SIM) mode. The ions monitored were 371, 473 and 488 for THC-COOH and 374 and 476 for D_3-THC-COOH.

Result: The method is validated according to the SWGTOX Standard Practices for Method Validation in Forensic Toxicology using spiked urine samples. The linearity was achieved for concentrations of THC-COOH ranged from 5 to 1200 ng/ml with the correlation coefficient (r) obtained at $\geq$ 0.9990. The limit of detection (LOD) and limit of quantitation (LOQ) were determined to be 5 ng/ml. Precision and accuracy studies of THC-COOH were performed at spiked concentrations of 15, 200, 600 and 1200 ng/ml. The within-run and between-run precisions were found to have coefficient of variations (CVs) ranged from 0.2 % to 4.6 %, and their accuracy ranged from 7.5 % to 9.4 %. No significant carryover of THC-COOH was observed for concentrations up to 1200 ng/ml. The extraction recoveries for THC-COOH using the Clean Screen XCEL® II extraction columns ranged from 63.0 % to 69.1 % for concentrations at 15, 200 and 600 ng/ml. The interferences studies and the application of this method in the detection of THC-COOH in the cannabis abusers urine samples will be discussed.

Conclusion: An automated solid phase extraction method for the detection and quantification of THC-COOH in urine by GC/MS was successfully developed using the Clean Screen XCEL® II performed in an automated solid-phase extraction system.

Keywords: Urine Analysis, Solid Phase Extraction, Delta-9-THC-COOH

P116
THC and THC-COOH in Oral Fluid: Immunoassay and LC-MS/MS

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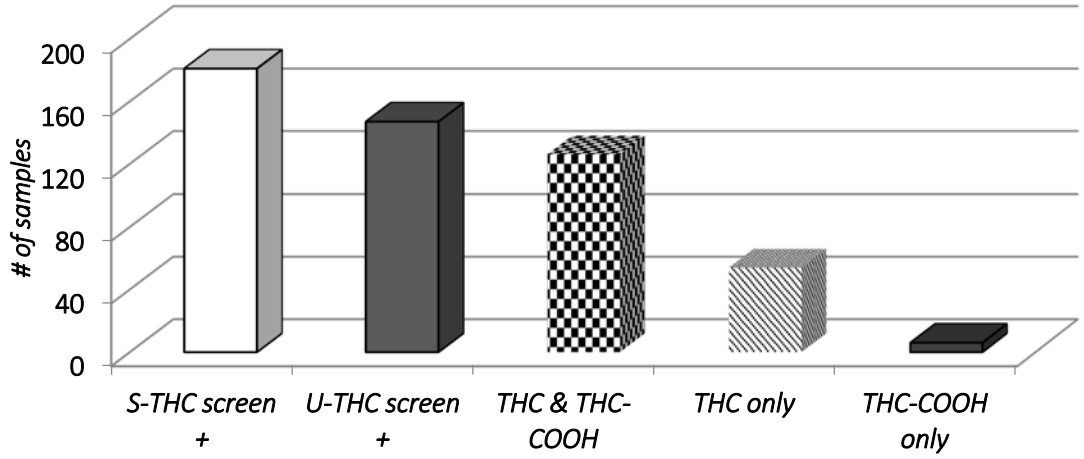
Introduction: Oral fluid (OF) is increasingly being analyzed for marijuana and its metabolites in many areas of drug testing. The presence of the metabolite THC-COOH is likely to indicate marijuana intake, whereas the presence of THC indicates recent use and possible passive exposure. An immunoassay for THC-COOH with low cross-reactivity to the parent drug may be useful for workplace testing where identification of drug intake is imperative. A retrospective study was undertaken to assess the utility of screening for THC only, THC-COOH only, or both in oral fluid.

Objective: To characterize the presence of THC and THC-COOH in OF using two separate immunoassay screens and LC-MS/MS confirmation

Method: ELISA results from two different assays in 187 OF specimens confirmed positive for THC and/or THC-COOH were studied retrospectively. The first ELISA targeted THC (Cut-off: 4ng/mL; S-THC); the second targeted the metabolite THC-COOH (Cut-off: 50pg/mL; U-THCA). Positive screens were confirmed by LC-MS/MS (THC LOQ: 2ng/mL: THC-COOH LOQ: 20pg/mL).

Result: *THC:* From 187 samples, 181 screened positively by S-THC; 179 were confirmed (98.8%). The two "false positives" contained THC-COOH at 34 and 208pg/mL. The 6 specimens screening negatively were positive on the ELISA U-THCA screen: 5 of those confirmed positively for THC-COOH and one sample had both THC (3ng/mL) and THC-COOH (139pg/mL).
THC-COOH: From 187 samples, 147 screened positively by U-THCA; 127 were confirmed (67.9%). All 20 samples not confirming for THC-COOH contained THC (2-285ng/mL). Of the 40 samples which initially screened negatively, 7 contained THC-COOH (28-150pg/mL; two ≤50pg/mL); all except one contained THC, which was the reason they were identified for confirmation (2-38 ng/mL). One sample which screened positively for THC and negatively for THC-COOH contained THC-COOH (34pg/mL). Regardless of the screening result, 67.3% had both THC and THC-COOH present; 28.8% contained THC only; and 3.7% contained THC-COOH only.



Discussion: Screening with only S-THC provided the greatest number of positive results (96.7%). Of those, 98.8% confirmed positively for THC using LC-MS/MS. The other six specimens (3.2%) were true positives and would not have been identified without the additional highly sensitive U-THCA assay. Screening with only U-THCA identified 147 positives (78.6%): THC-COOH confirmed in 86.3% using LC-MS/MS; the other 20 all confirmed for THC but not for the metabolite.

Summary: Screening for only THC-COOH identifies a low number of true positive saliva samples which would be missed by assays targeting only THC; however implementation of an additional assay may not be cost-effective in a high volume laboratory, especially if recent use is the reason for testing and not historical marijuana intake.

Keywords: THC-COOH, Immunoassay, Oral Fluid

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Comparison of the Randox® Evidence Drugs of Abuse Custom Array VIII Biochip with Accurate Mass Screening II: Stimulants

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Introduction: Blood specimens collected in suspected DUID cases in the State of Michigan are routinely screened for drugs by the Michigan State Police using a Randox® Evidence Analyzer and a Drugs of Abuse Custom Array VIII Biochip employing chemiluminescent immunoassay technology. The custom chip is imbedded with 14 different antibodies to desired target analytes in discrete testing regions. As part of a workload reduction project, specimens that screened positive for one or more analytes on the biochip were sent to NMS Labs for analysis by liquid chromatography accurate mass screening and confirmation of presumptive positive findings.

Objective: The aim of this study was to compare the results obtained between the custom biochip stimulant assays (amphetamine (AMP), methamphetamine (MAMP) and benzoylecgonine (BZE)) with LC-TOF accurate mass screening and LC-MS/MS or GC/MS confirmation.

Method: Randox® Biochip screening was targeted at 20 ng/mL (d-amphetamine) 40 ng/mL (d-methamphetamine) and 20 ng/mL (benzoylecgonine) for the AMP, MAMP and BZE assays, respectively. The cross-reactivities for the major analytes are summarized in Tables 1 and 2. The LC-TOF accurate mass screening and MS confirmations had cutoffs as noted in Table 3. Only cases which tested positive above the LC-TOF decision point were confirmed.

Table 1: Randox Biochip % Cross-Reactivity for the amphetamine and methamphetamine assays (1)

Assay	d-amp	dl-amp	d-mamp	MDMA	MDEA	MDA	Phentermine
AMP (1)	100	29.5	<0.01	0.4	1.4	426	35.2
MAMP (1)	0.6	0.5	100	38	2.1	0.6	<0.4

(1) Cross-reactivity data is based on commercial DoA I+ chip

Table 2: Randox Biochip % Cross-Reactivity for the benzoylecgonine assay (1)

BZE	COC	CE	EME	NCOC
100	84.8	56.8	<0.01	0.28

(1) Cross-reactivity data is based on commercial DoA I+ chip

Table 3: LC-TOF Decision Points and LC-MS/MS and GC/MS Reporting Limits (ng/mL)

Method	AMP	MAMP	MDMA	MDA	Phentermine	BZE	COC	CE
LC-TOF	10	10	10	10	50	100	20	20
LC-MS/MS	5	5	5	5	10	-	-	-
GC/MS	-	-	-	-	-	50	20	20

Result: A total of 1858 blood specimens were tested. Table 4 summarizes the data obtained by the biochip assays and the LC-TOF screen. For AMP and MAMP the values in parenthesis represent the results obtained after correcting for the differences in screening decision points between the biochip and LC-TOF for the target compounds as determined by LC-MS/MS. These are used to calculate the results. For the BZE assay, 7/8 cases that were positive by biochip had LC-TOF results that showed evidence of BZE below the decision point but they were not confirmed and therefore not adjusted in the data.

Table 4: Results: Biochip and LC-TOF screen (parenthetical data corrected for differences in screening decision points).

AMP			MAMP			BZE		
	LCTOF(+)	LCTOF (–)		LCTOF(+)	LCTOF(–)		LCTOF(+)	LCTOF (–)
Chip +	178	11	Chip +	64	22	Chip +	112	8
Chip –	70 (17)	1599	Chip –	18 (4)	1754	Chip –	0	1738

For the AMP assay, total positives included 5 MDA and 11 phentermine cases consistent with cross-reactivity of the assay for these drugs. There were also 5 confirmed MDMA positive cases. However, the MAMP assay was only positive for 2 of these cases, even though 4 out of 5 were above the expected cutoff for the assay based on cross-reactivity data.

Conclusion: A total of 1858 blood specimens analyzed on a Randox® Evidence Analyzer with a Drugs of Abuse Custom Array VIII Biochip were evaluated for stimulant drugs (AMP, MAMP and BZE). The percent agreement between the AMP, MAMP and BZE assays and the LC-TOF screen was 98.4%, 98.7%, 99.5%, respectively. The specificity and sensitivity for the assays were as follows: AMP (99.3%, 91.2%), MAMP (98.7%, 94.1%) BZE (99.5%, 100%).

Keywords: Screening, Randox®, LC-TOF

P118 ***"NIJ Funded"***

Analysis of a Marijuana E-Liquid for Use in Electronic Cigarettes Using Direct Analysis in Real Time- Mass Spectrometry, Gas Chromatography-Mass Spectrometry, and High Performance Liquid Chromatography-Tandem Mass Spectrometry

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Introduction: With the growing popularity of electronic cigarettes, use of these devices has expanded from nicotine delivery to other drugs. E-liquids are formulations composed of propylene glycol and/ or glycerin, flavoring components, and drug. They are aerosolized by the electronic cigarette for inhalation. The ease of customization of electronic cigarettes and e-liquids present potential public health and criminal justice issues.

Objective: Presented is the analysis of an e-liquid, Liberty Reach, for marijuana cannabinoids, terpenes and glycols. Liberty Reach was advertised to contain 69.1% ∆9-tetrahydrocannabinol (THC) and 1% cannabidiol (CBD). The analysis was performed using a JEOL Direct Analysis in Real Time-AccuTOF™ Mass Spectrometer (DART-MS) (Peabody, MA), an Agilent Gas Chromatography-Mass Spectrometer (GC-MS) (Santa Clara, CA), and an Applied Biosystems Tandem Mass Spectrometer (MS/MS) attached to a Shimadzu SCL High Performance Liquid Chromatography system (HPLC) (Kyoto, Japan).

Method:

DART-MS: The Liberty Reach was screened for cannabinoids, marijuana terpenes, and glycols using the DART-MS controlled by Mass Center software version 1.3.4 m with Orifice-1 operated at 300 °C in function switching mode at 20, 60, and 90 V. Accuracy of the data was evaluated by a mass difference of ±5 mmu.

GC-MS: The Liberty Reach was qualitatively analyzed for 42 different marijuana terpenes and 8 glycols using a 6890N/5973 GC-MS with a Restek Rxi-624Sil MS column (30 m x 0.25 mm, 1.4 micron) (Bellefonte, PA) controlled by ChemStation Software version D 01.02.16. The oven temperature program was set at 60 °C and held for 0.1 min, then ramped to 300 °C at 12.5 °C/min, then held for 3 min.

HPLC-MS/MS: The Liberty Reach was analyzed for cannabinoids using a 3200 QTRAP HPLC-MS/MS with a Zorbax Eclipse XDBC18 column (4.6 × 75 mm, 3.5 micron, Agilent Technologies) controlled by Analyst 1.4.2 software. Multiple reaction monitoring (MRM) transition ions were: *m/z* 315>193 for THC/CBD/Cannabichromene (CBC); *m/z* 317>193 for Cannabigerol (CBG)*; m/z* 311>293 for Cannabinol (CBN); *m/z* 287.2>165.2 for Cannabidivarin (CBDV); *m/z* 331.2>193.1 for 11-Hydroxy-Δ9-tetrahydrocannabinol (11-OH-THC); *m/z* 345.1>299.2 for 11-Nor-9-Carboxy-THC (THCCOOH); m/z 359>341 for Tetrahydrocannabinolic acid-A (THCA-A); *m/z* 318>196 for THC-d3/CBD-d3; *m/z* 334>196 for 11-OH-THC-d3, and *m/z* 348>302 for THCCOOH-d3; m/z 290>168 for CBN-d3.

Result:

Glycols and Marijuana Terpenes identified: Propylene glycol and thirteen marijuana terpenes: α-pinene, β-myrcene, β-pinene, limonene, (1R)-endo-(+)-fenchyl alcohol, linalool, α-terpineol, borneol, β-caryophyllene, α-humulene, guaiol, (+)-cedrol, and α-bisabolol were identified in the Liberty Reach.

Cannabinoids identified: Seven cannabinoids were identified and quantitated in the Liberty Reach (v/v): THC (42%), CBD (0.5%), THCCOOH (0.025%), CBN (0.36%), CBC (0.72%), CBG (0.64%), THCA-A (5.6%).

Conclusion/Discussion: The concentration of the THC, 42.6% (v:v), and CBD, 0.5% (v:v), was different from the labeled contents of the Liberty Reach, THC at 69% and CBD at 1%. Five additional cannabinoids were identified and quantitated. Also Propylene glycol and thirteen marijuana terpenes were identified in the e-liquid. Although the THC content was lower than labeled, 42.6% THC (v/v), e-liquids may well warrant public health concerns about the use of marijuana in electronic cigarettes.

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Keywords: THC, E-Liquid, Electronic Cigarettes

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Screening for Synthetic Cannabinoids via Non-Targeted Liquid Chromatography-Quadrupole/Time-of-Flight High-Resolution Mass Spectrometry

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Background: Despite legislative efforts, new synthetic cannabinoids continue to emerge, challenging clinical, forensic and workplace urine drug testing laboratories. Immunoassays are unable to keep pace with constantly emerging compounds, necessitating alternative urine screening approaches. Liquid chromatography-quadrupole/time-of-flight high-resolution tandem mass spectrometry (LC-Q/TOF) provides a sensitive, specific and updateable screening alternative to synthetic cannabinoid immunoassay screening. Current LC-Q/TOF technology requires longer acquisition time and more labor-intensive data review than immunoassay screening; however, non-targeted LC-Q/TOF methods are easily updated for new analytes and allow retrospective analysis for newly emerging compounds.

Objective: We recently developed a sensitive LC-Q/TOF synthetic cannabinoid screening method employing non-targeted acquisition for 48 synthetic cannabinoid metabolites from 22 synthetic cannabinoid families (5-fluoro-AB-PINACA, 5-fluoro-AKB48, 5-fluoro-PB-22, AB-PINACA, ADB-PINACA, AKB48, AM2201, BB-22, JWH-018, JWH-019, JWH-073, JWH-081, JWH-122, JWH-200, JWH-210, JWH-250, JWH-398, MAM2201, PB-22, RCS-4, UR-144 and XLR11). We evaluated method performance by analyzing 29 authentic urine specimens collected during Swedish petty drug investigations (n=27), from one Swedish drug treatment center patient (n=1) and from one Swedish correction facility investigative case (n=1). All 29 specimens were analyzed via two independently developed LC-Q/TOF methods targeting the same synthetic cannabinoid families.

Method: Method #1: 0.6mL urine was hydrolyzed at room temperature for 20 min with 4.2U *E. coli* β-glucuronidase before salting-out, liquid-liquid extraction with 0.4mL acetonitrile and 0.2mL 10M ammonium acetate. Method #2: 0.25mL urine was hydrolyzed for 1h at 55°C with 625U Red abalone β-glucuronidase before supported liquid extraction. Both methods employed non-targeted MS/MS acquisition on an Agilent 6540 (method #1, runtime=15 min) or on a SCIEX TripleTOF (method #2, runtime= 15 min) LC-Q/TOF instrument. Method #1 limits of detection (LOD) were 5µg/L for all analytes except BB-22 3-carboxyindole, PB-22 3-carboxyindole and 5-fluoro-PB-22 3 carboxyindole (100µg/L); method #2 LOD were 0.25-5µg/L, except for PB-22 N-hydroxypentyl-3-carboxyindole and PB-22 N-pentanoic-3-carboxyindole with limits of 10 and 20 µg/L, respectively.

Result: There was good agreement on the identification of synthetic cannabinoids by both LC-Q/TOF methods for all 29 cases; however, additional synthetic cannabinoids were observed with method 2 in 11/29 cases. 5-fluoro-AKB48 (n=12), 5-fluoro-PB-22 (n=2), AB-PINACA (n=2), AKB48 (n=8), AM2201 (n=2), BB-22 (n=7), JWH-018 (n=2), and JWH-073 (n=2) were identified by both methods. Method 2 additionally identified: JWH-018 (n=1) and UR-144 (n=4) with 5-fluoro-AKB48; AKB48 (n=1) and JWH-019 (n=1) with 5-fluoro-PB-22; JWH-019 (n=1), JWH-122 (n=1) and JWH-398 (n=1) with AB-PINACA; JWH-018 (n=1) and UR-144 (n=1) with AKB48; and AKB48 (n=1) with BB-22.

Conclusion: These data support implementation of non-targeted LC-Q/TOF screening as an alternative to immunoassays for synthetic cannabinoids urine screening. Increased method 2 analyte sensitivities could account for additional analytes identified by method #2 that were not observed with method #1. Low limits of detection are important for synthetic cannabinoid identification due to low urinary concentrations.

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Keywords: Synthetic Cannabinoids, Urine, High-Resolution Mass Spectrometry, Screening

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Development of a Synthetic Cannabinoid Receptor BioAssay as a Urine Screening Method

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Background: Cannabinoid receptor 1 (CB_1) and CB_2 are membrane bound G-protein coupled receptors (GPCRs). When activated by an agonist, CB_1 and CB_2 stimulate an inhibitory G-protein (G_i) which inhibits adenylate cyclase and decreases cyclic adenosine monophosphate (cAMP) concentrations in cells. CB_1 receptors mediate many of the central nervous system (CNS) effects associated with exogenous cannabinoid use. CB_2 receptors are associated with the immune system and the peripheral nervous system. Synthetic cannabinoids were originally synthesized as research compounds to study the endocannabinoid system, but recently have become drugs of abuse. Because of the large number and constantly changing structures of these drugs, it is difficult to know which compound(s) are in abused products and forensic testing laboratories struggle with keeping detection methods up-to-date. As a consequence, more innovative screening methods need to be developed to aid in the identification of synthetic cannabinoids and their metabolites.

Objective: Develop a cannabinoid receptor bioassay to screen urine samples for synthetic cannabinoids.

Method: 33 targeted urine samples were extracted for the bioassay and LC-MS/MS. Samples were extracted by SPE for the bioassay. Briefly, CHO cells expressing CB_1 and CB_2 were plated in 96-well ½ area plates and stimulated with forskolin. Changes in cAMP in the cells were measured with an optimized LANCE® Ultra cAMP Assay. For confirmation analysis, a separate aliquot of the urine sample was prepared using an alternate SPE method. Samples were analyzed on an LC-MS/MS using an AB Sciex 4500 in selected multiple reaction monitoring (SMRM) mode. The method detects over 30 synthetic cannabinoid metabolites spanning 1st thru 4th generation compounds.

Result: Thirty-three urine specimens were selected for comparison of bioassay vs. LC-MS/MS. Twenty of the specimens were selected because they were suspected synthetic cannabinoid positives, 6 specimens were selected for additional LC-MS/MS analysis because they produced aberrant bioassay results, e.g. outside the dynamic range and 7 specimens were anticipated negatives for synthetic cannabinoids. These same 7 samples tested negative on both platforms. None of the 6 samples that were outside of the dynamic range of the bioassay were positive via LC-MS/MS. The results from the 33 specimens analyzed on both platforms are summarized the tables below.

Table 1. Summary of Bioassay results.

Bioassay		
Negative	Non-Negative	Outside Dynamic Range
7	20	6

Table 2. Summary of LC-MS/MS results.

LC-MS/MS		
Negative	Positive	Indeterminate
7	9 (6 quantitative, 3 qualitative)	17

Conclusion/Discussion: The bioassay is a sensitive method to screen urine samples for the presence of compounds that specifically bind to the cannabinoid receptor. However, the availability of reference material can be a limiting factor in identification and confirmation of these compounds. When samples are identified as non-negative with the bioassay and fail to confirm with LC-MS/MS, additional structural elucidation experiments may be undertaken to identify reactive compounds. This approach will better aid forensic laboratory scientists in monitoring new synthetic compounds abused and direct analysis to identify necessary major metabolites.

Keywords: Synthetic Cannabinoids, Receptor Bioassay, Screening Assay

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Heroin-Related Overdoses in Alabama: Heroin and Fentanyl – A Deadly Combination

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Background: Heroin (diacetylmorphine) is a semi-synthetic opiate processed from morphine, which is derived from the opium poppy plant. It is typically sold as a white or brownish powder or as a sticky black substance, commonly called Black Tar Heroin. Heroin can be injected intravenously (IV), smoked, or snorted. The major metabolite of heroin is 6-monoacetylmorphine (6-MAM), and this is used as a marker for heroin use. Heroin has been in the news frequently in the last few years, and the media has reported on heroin overdoses, drug seizures by law enforcement, and potential solutions to the heroin problem. There have been reports of cases involving both heroin and fentanyl, a synthetic opioid commonly prescribed to terminally ill patients.

Objective: To investigate the prevalence of heroin overdoses in Alabama over the last six years, the frequency of other drugs commonly seen in these cases, and the demographics of these heroin users. Lastly, three interesting case studies involving heroin overdoses were characterized.

Method: Death cases were evaluated over a six year period (2009-2014) based on receipt date. All cases were screened at the Alabama Department of Forensic Sciences (ADFS) by ELISA using Tecan Evo75 with Immunalysis reagents or Randox Evidence Analyzer. Confirmation of heroin (6-MAM) was performed by liquid-liquid extraction using ToxiLab A for urine, or solid-phase extraction using Clean Screen SPE with GC/MS in blood, urine, and vitreous humor. ADFS serves all counties in Alabama except for Jefferson County. In collaboration with the Jefferson County Coroner's Office, data was provided regarding heroin overdoses in Jefferson County for the same time period. In those cases, urine was screened for common drugs of abuse using EMIT, and quantitation was done in blood using GC/MS. Studies show that a low codeine-to-morphine ratio (<1) is suggestive of a heroin overdose. Cases where 6-MAM was not identified but had a codeine-to-morphine ratio <1, as well as supporting evidence of possible heroin use, were considered for the same time period.

Result: The number of heroin overdoses increased approximately 15-fold from 2009 to 2014, with a 4.5-fold increase between 2013 and 2014. There has been a noticeable trend of fentanyl being taken concurrently with heroin. Seventeen percent of heroin cases contained fentanyl in 2014. Jefferson County and ADFS reported a total of 18 heroin plus fentanyl cases in 2014, but neither agency had any cases in the previous five years. The average age of heroin users was 35 years old (range 17-68). Seventy-one percent of the users were male and 29% were female. Ninety percent of users were Caucasian, 9% were African-American, and 1% were Hispanic. Geomapping indicated a large concentration of heroin users in the central to northern region of the state, including the largest city of Birmingham. There was a significant incidence of cases that had a very low codeine-to-morphine ratio (<0.3).

6-MAM Present in Cases from 2009 – 2014			
Year	Number of Cases at ADFS	Number of Cases in Jefferson County	ADFS + Jefferson County
2009	9	19	28
2010	3	12	15
2011	9	30	39
2012	9	63	72
2013	12	30	42
2014*	46	144	190
Grand Total	88	328	416

*Addition of 6-MAM to SPE opioid analysis.

Conclusion: Heroin abuse increased significantly in Alabama over the last six years, particularly in 2014. Reasons for the increase may include higher purity heroin being distributed which makes it easier to snort, the reformulation by pharmaceutical companies of drugs like oxycodone, that make it more difficult to melt down and inject intravenously, and the movement away from expensive prescription drugs toward the less expensive heroin. Heroin combined with fentanyl is more dangerous than either drug on its own. Fentanyl is being illicitly manufactured and mixed with heroin, or it is being misrepresented and sold as heroin. This may account for the trend towards identifying the two drugs concurrently. Our future aim is to develop and validate an improved opioid method using QTOF technology to include morphine, 6-MAM, morphine-glucuronides and fentanyl, so that we may determine the concentration of these drugs in all specimens.

Keywords: Heroin, Fentanyl, Overdoses

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