Abstracts Of Platform Presentations

S01

Cannabinoids Disposition in Blood Following Controlled Cannabis Administration by Volcano® Vaporizer

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Introduction: Cannabis is the most widely used illicit drug, taken for recreational purposes or medicinally. Smoking is the most common cannabis administration route, due to rapid drug delivery and ability to titrate dose. Inhaling combustion byproducts is carcinogenic. Vaporizers gain popularity as alternative inhalation routes, volatilizing desired cannabinoids while remaining below combustion temperature. They also minimize side-stream loss and heat-degradation of psychoactive Δ^9-tetrahydrocannabinol (THC), providing a more efficient administration route. THC and metabolites' disposition after inhalation is not fully characterized in human blood.

Objective: To evaluate cannabinoids disposition in blood following vaporized controlled cannabis administration.

Method: Placebo, low, and high-potency (0, 2.9, and 6.7% THC, respectively) bulk cannabis material (0.5 g) was ground and vaporized in the Volcano® Medic vaporizer (Storz & Bickel, Tuttlingen, Germany) at 210°C per manufacturer instructions. Participants inhaled cannabis vapor over 10 min. Blood was drawn -0.75, 0.17, 0.42, 1.4, 2.3, 3.3, 4.8, 6.3, and 8.3 h after dosing commenced and stored at -20°C until analysis within 1 month of collection. THC, 11-hydroxy-THC (11-OH-THC), carboxy-THC (THCCOOH), THC-glucuronide (THC-glu), THCCOOH-glucuronide (THCCOOH-glu), cannabidiol (CBD), and cannabinol (CBN) were quantified by liquid chromatography-tandem mass spectrometry (LCMSMS) according to a previously-published method. Limits of quantitation were 1.0 (THC, 11-OH-THC, THCCOOH, CBD, and CBN), 5.0 (THCCOOH-glu), and 0.5 (THC-glu) ng/mL, respectively. Maximum concentration (C_{max}), time of maximum concentration (t_{max}), time of first detection (t_{first}), and time of last detection (t_{last}) were assessed, excluding sessions where no analyte was detected from t_{max}, t_{first}, and t_{last} calculations.

Results: Five healthy adult cannabis smokers (4 M, 1 F; ages 22-38; smoked cannabis 1 day/month-3 days/week) provided written informed consent and completed this IRB-approved, double-blind, within-subject cannabis administration study. Median [range] cannabinoid blood C_{max} (ng/mL), t_{max}, t_{first}, and t_{last}(h) were:

	THC	11-OH-THC	THCCOOH	THC-glu	THCCOOH-glu	CBD	CBN
C_{max}							
Low	58.2 [24.3-63.5]	4.3 [2.6-9.1]	21.4 [17.7-41.8]	ND	35.8 [22.1-55.9]	ND	2.3 [0-3.3]
High	63.4 [30.6-137]	6.5 [5.0-12.8]	42.8 [29.1-50.4]	0 [0-0.8]	49.4 [43.2-85.5]	1.5 [0-3.6]	2.0 [0-3.0]
t_{max}							
Low	0.17 [0.17-0.17]	0.17 [0.17-0.42]	0.42 [0.17-1.4]	--	2.3 [1.4-4.8]	--	0.17 [0.17-0.17]
High	0.17 [0.17-0.17]	0.17 [0.17-0.17]	0.42 [0.17-0.42]	0.42 [0.42-0.42]	2.3 [1.4-4.8]	0.17 [0.17-0.17]	0.17 [0.17-0.17]
t_{first}							
Low	0.17 [-0.75-0.17]	0.17 [0.17-0.17]	-0.75	--	-0.75 [-0.75-0.17]	--	0.17 [0.17-0.17]
High	0.17 [0.17-0.17]	0.17 [0.17-0.17]	-0.75	0.42 [0.42-0.42]	-0.75 [-0.75-0.17]	0.17 [0.17-0.17]	0.17 [0.17-0.17
t_{last}							
Low	8.3 [3.3-8.3]	2.3 [1.4-2.3]	8.3 [8.3-8.3]	--	8.3 [8.3-8.3]	--	0.17 [0.17-0.17]
High	8.3 [4.8-8.3]	4.8 [2.3-8.3]	8.3 [8.3-8.3]	0.42 [0.42-0.42]	8.3 [8.3-8.3]	0.17 [0.17-0.17]	0.17 [0.17-0.17]

C_{max} was dose-dependent for all analytes except CBN. THC peaked immediately and was still detectable 8.3 h later at both doses. THC-glu and CBD were only detected at 0.42 h in 2 and 3 individuals (respectively) following the high dose. THCCOOH was detected throughout the time course, but peaked <1 h post-inhalation.

Conclusion: Vaporizer administration produced comparable cannabinoid pharmacokinetic profiles to those observed after smoked cannabis, verifying its utility for effective cannabis administration.

Keywords: Cannabis, Blood, THC, Pharmacokinetics, Vaporizer

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S02

Cannabimimetic Behavioral Effects of the Synthetic Cannabinoid, CP47,497 are Mediated by CB1 Receptors

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Introduction: Our proposed research involves investigating the pharmacological and toxicological consequences of synthetic cannabinoid use. Synthetic cannabinoids continue to be abused as a legal alternative to marijuana and currently pose a threat to public health and safety as increasing numbers of undesired side effects and fatalities have been reported. Since these compounds were originally synthesized as research tools to study the endogenous cannabinoid system, preclinicalstudies examining the effects of synthetic cannabinoids are insufficient to date.

Objective: We sought to characterize*in vivo*effects of the prototypical synthetic cannabinoid, CP47,497 in mice using established preclinical models, tetrad and drug discrimination. Our previous studies have demonstrated CP47,497 acts in a dose and time dependent manner, implicating a receptor mediates the drug's actions. Resultantly, our second objective was to investigate whether CP47,497's cannabimimetic effects are mediated through cannabinoid type 1 receptors (CB1R) using complimentary pharmacologic and genetic approaches.

Method: Cannabimimetic subjective effects in wild-type ($CB1^{+/+}$) and knock-out ($CB1^{-/-}$) mice were evaluated using the well-established tetrad behavioral model consisting of four outcome measures sensitive to the chief psychoactive cannabinoid present in marijuana, delta-9-tetrahydrocannabinol (THC): catalepsy, antinociception, hypothermia, and decreases in spontaneous locomotor activity. Furthermore, to assess the abuse potential of CP47,497, a drug discrimination paradigm was employed using mice trained to discriminate 5.6 mg/kg THC from vehicle. Interestingly, the CB1R antagonist, rimonabant, was able toattenuate behavioral effects of CP47,497, but was more potent in blocking catalepsy and antinociception than preventing hypothermia and locomotor depressant effects. Importantly, all cannabimimetic effects of CP47,497 were abolished in $CB1^{-/-}$mice. CP47,497 fully substituted for THC in mice trained to discriminate 5.6 mg/kg THC from vehicle; demonstrating a potency 5 times that of THC. Concomitant with behavioral experiments, validation experiments were successfully completed for optimization of a Liquid Chromatograph/Tandem Mass Spectrometer (LC/MS/MS) analytical method for detection and quantitation of synthetic cannabinoids in whole blood. Immediately following behavioral testing, blood and tissue were banked for drug quantification and will be analyzed on an Applied Biosystems LC/MS/MS interface utilizing electrospray ionization and selective ion monitoring after acetonitrile liquid-liquid extraction. Future studies will correlate cannabimimetic behavior to levels of CP47,497 in blood and brain, permitting a better understanding of how behavior is affected following synthetic cannabinoids drug exposure.

Results: Experiments using $CB1^{-/-}$mice corroborate the results of our antagonism studies and provide the first known evidence that CP47,497 produces THC-like effects through a CB1R-mediated mechanism of action*in vivo*. Given that CP47,497 achieves its behavioral effects through CB1 receptors and elicits dose-dependent cannabimimetic effects that are markedly (i.e. 7-9 fold) more potent than THC, these data are consistent with the large number of synthetic cannabinoids abusers presenting with severe and toxic side effects. Furthermore, CP47,497 was more potent than THC in drug discrimination studies, supporting an enhanced potential for abuse when compared to marijuana.

Conclusion: These data begin to address themechanisms that underlie risks associated with synthetic cannabinoid use. Consequently, ongoing research will investigate drug disposition in mice and assess tolerance and dependence liability after repeated synthetic cannabinoid exposure.

Keywords: Synthetic Cannabinoid, CP47,497, Cannabimimetic Behavior

S03

Risk for Neurobehavioral Disinhibition in Prenatal Methamphetamine-Exposed Young Children with Positive Hair Toxicology Results

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Introduction: Prevalence of illicit amphetamine stimulant use is second only to cannabis worldwide. While initiation of methamphetamine use remains relatively stable, methamphetamine treatment admissions and manufacturing seizures continue to be a significant problem. Methamphetamine is the primary substance used by pregnant women in drug treatment facilities in the US.Despite significant use among pregnant women, little is known about the developmental and behavioral impact of prenatal methamphetamine exposure (PME). Neurobehavioral disinhibition is a set of co-occurring problems including poor self-regulation, cognitive impairment, disruptive behavioral disorders, and anxiety and affective disorders. Children and adolescents exhibiting these disinhibitory problems often have consistently poor outcomes as adults.

Objective: The objective of this study was to evaluate effects of prenatal and postnatal methamphetamine exposure on child neurobehavioral disinhibition at 6.5 years.

Method: Mother-infant pairs were enrolled in the Infant Development, Environment, and Lifestyle (IDEAL) Study in Los Angeles, Honolulu, Tulsa and Des Moines. PME was determined by maternal self-report and/or positive meconium results. At the 6.5-year follow-up visit, a 3 cm proximal hair segment was collected. Twenty milligrams was analyzed for drugs with limits of quantification (LOQ, pg/mg) of 51 for amphetamine, 68 methamphetamine, 58 3,4-methylenedioxymethamphetamine (MDMA), 52 3,4-methylenedioxyamphetamine (MDA), 38 cocaine, 4 benzoylecgonine, 36 norcocaine, 11 cocaethylene, 10 Δ^9-tetrahydrocannabinol, 51 nicotine, and 51 cotinine. Child behavioral and executive function test scores were aggregated to evaluate child neurobehavioral disinhibition. Linear regression models assessed the impact of prenatal methamphetamine, postnatal substances and combined exposures on the child's neurobehavioral disinhibition aggregate score. Caregiver substance intake also was collected and compared with child hair toxicology results.

Results: 264 children's hair specimens were evaluated. No hair specimen was positive for MDMA, MDA, norcocaine, or cocaethylene. Significantly more children with PME (n=133) had hair positive for methamphetamine/amphetamine (27.1% versus 8.4%) and nicotine/cotinine (38.3% versus 25.2%) than children with no PME (n=131). Overall, no significant differences in analyte hair concentrations were noted between groups. Significant differences in behavioral and executive function were observed between children with and without PME. No independent effects of postnatal exposure to methamphetamine or tobacco were noted. However, a significant additional risk of neurobehavioral disinhibition, indicated by poor behavioral and executive functions, was seen in children with combined PME and postnatal methamphetamine and/or tobacco exposure. This study was unique as self-reported caregiver substance use information was collected simultaneously with the child's hair. Agreement between caregiver reported methamphetamine use and hair results among both groups of children was poor. A significantly higher prevalence of self-reported tobacco use from caregivers of PME children was seen as compared to children with no PME. Average cigarette use among caregivers reporting current tobacco use was significantly higher among children with positive hair as compared to those with negative hair in both groups.

Conclusion: These data demonstrate child hair drug testing is a non-invasive means to assess additional risk for neurobehavioral disinhibition. PME prevention may lead to reduced risk of neurobehavioral disinhibition, and in children with PME, prevention of postnatal drug exposure in early childhood could result in improved executive and behavior functions.

Keywords: Prenatal Methamphetamine Exposure (PME), Neurobehavioral Disinhibition

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S04

The Emerging of New Synthetic Cannabinoids and Their Binding Affinities at the Cannabinoid CB_1 and CB_2 Receptors

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Introduction: To counter the spread of the many analogs of psychoactive substances, the Pharmaceutical Affairs Law in Japan was amended in 2006 to establish a new category; "Designated Substances" in order to more promptly control these drugs. However, new analogs of controlled substances, especially synthetic cannabinoids, appeared one-by-one. To avoid a cat-and-mouse game, a comprehensive system (generic definition) for designating naphthoylindole-type synthetic cannabinoids, having particular substituents, was introduced into the "Designated Substances" in 2013.

Objective: The distribution of synthetic cannabinoids before and after their control in Japan was investigated for the last four years.Moreover, their binding affinities at the cannabinoid CB_1 and CB_2 receptors were evaluated.

Method: 1087 products (blended herbs or powders) were collected via the Internet from September 2009 to March 2013. Their MeOH extracts were analyzed using GC-EI-MS and LC-ESI-MS. The identification of unknown compounds was mainly done by NMR and TOFMS analyses. The evaluation of the IC_{50} values of 15 synthetic cannabinoids, which had newly emerged and had not yet had their pharmacological activities reported, was based on the competitive interaction between a labeled ligand ([^{3}H] CP-55,940) and an analyte for the human cannabinoid CB_1 and CB*2* receptors binding sites.

Results: As of March 2013, at least 59 kinds of synthetic cannabinoids have been identified from the products. In 2009, only cyclohexylphenols (e.g. cannabicyclohexanol) and naphthoylindoles (e.g. JWH-018) were found. However, following the control of these compounds, the cyclohexylphenols disappeared from the illegal drug market and various analogs of the naphthoylindoles (e.g. JWH-081, JWH-210 and AM-2201), phenylacetylindoles (e.g. JWH-203) and benzoylindoles (e.g. AM-694) were widely distributed. Other compounds not having the four structures described above had never been detected prior to 2011 and 63% of the compounds detected from 2009 to 2011 were the naphthoylindoles. MAM-2201 was the most frequently detected in 2012. In this same year some health damage, possibly caused by this compound, was also reported. In the second half of fiscal 2012, new types of synthetic compounds such as carboxyamides and quinolinyl carboxylates dramatically increased (71 % of the detected compounds). In particular, after the official announcement of the generic definition in November 2012, the naphthoylindoles were rarely detected. The quinolinyl carboxylates, PB-22 and 5-fluoro PB-22, were the most detected in the products obtained in early 2013.

Among the 15 compounds measured in this study, the binding affinities of MAM-2201 at the CB_1 and CB_2 receptors were the highest and 9.5 and 1.6 times higher than those of JWH-122. The *N*-fluoroalkyl analogs of an indole moiety tended to have higher affinities as well as MAM-2201/JWH-122. The carboxyamides (e.g. JWH-018 adamantyl carboxamide) and the quinolinyl carboxylates (e.g. PB-22) showed almost similar affinities to that of JWH-018.

Conclusion: It was shown that most of recent emerging synthetic cannabinoids have high CB_1/CB_2 receptor binding affinities and potential serious health damage may be expected.To avoid health problems and abuse caused by new designer drugs, we have to continuously monitor the distribution of these products.

Keywords: New Psychoactive Substances, SyntheticCannabinoids, Receptor Binding Affinity

S05

Cannabinoid Receptor Potency of Synthetic Cannabinoids Present in Skyscraper: The Next Level

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Introduction: Synthetic cannabinoids have been at the forefront of the designer drugs trend for the last several years. Cannabinoid ligands exert pharmacodynamic effects by binding to cannabinoid receptors, resulting in modified neurotransmission. Cannabinoid receptors 1 (CB_1) and 2 (CB_2) are coupled to $G_{i/o}$ proteinsand when activated, inhibit adenylyl cyclase, and decrease intracellular cyclic adenosine monophosphate (cAMP). CB_1 receptors are present primarily in the central nervous system and mediate psychotropic effects of exogenous cannabinoids, while CB_2 receptors are responsible for immunomodulatory effects. Increased potency at CB_1 may explain synthetic cannabinoids' enhanced physiological and psychological effects compared to THC. Packages of "Skyscraper: the Next Level," an herbal incense blend, were obtained from law enforcement after multiple individuals who ingested the product exhibited psychotic symptoms and were hospitalized.

Objective: Our objectives were to determine the analytes present in "Skyscraper: The Next Level" and establish the potency of the detected synthetic cannabinoids at CB_1 and CB_2 receptors.

Method: Three packages of herbal material were received labeled "Skyscraper: The Next Level" in three varieties: mango, strawberry, and blueberry. Methanolic extracts from each flavored product were dried under nitrogen, reconstituted in mobile phase, screened with an Applied Biosystems QSTAR Elite Hybrid LC/MS/MS, and compared to reference standards of JWH-210, JWH-098, JWH-081, JWH-201, JWH-019, JWH-250, RCS-4, JWH-200, AM-2201, JWH-073, JWH-018, and UR-144. Synthetic cathinones were screened on an AB Sciex 3200 LC/MS/MS and compared to reference standards of Butylone, 3-Fluoromethcathinone, Methylone, Methedrone, Mephedrone, Methylenedioxypyrovalerone, and Naphyrone. Confirmation was conducted on an AB Sciex 4500 LC/MS/MS and three transitions were monitored (UR-144 312/214, 312/125, 312/144; AM-2201 360/155, 360/232, 360/127). Varying cannabinoid standard concentrations were incubated with Chinese hamster ovary (CHO) cells expressing human cannabinoid receptors and forskolin, a cAMP stimulator; changes in cAMP were measured. Analytical standards of UR-144 and AM-2201 were compared to THC, JWH-018, and HU-210 in the bioassay. Potency at the receptor was assessed by determining the concentration that caused 50% maximal effect (EC_{50}) for each synthetic cannabinoid observed in the products, as well as THC, JWH-018, and HU-210, which served as reference analytes.

Results: All three "Skyscraper" varieties screened non-negative for UR-144. Additionally, strawberry and mango varieties screened non-negative for AM-2201. Results were confirmed by LC/MS/MS. No presence of cathinones was noted. All compounds assessed produced a receptor-mediated response at CB receptors. Potency at CB_1 was HU-210 >JWH-018 >AM-2201 >THC >UR-144. UR-144 was substantially more potent at CB_2 compared to CB_1.

Conclusion: Although UR-144 displayed decreased potency at CB_1 compared to CB_2, its presence in herbal incense material has increased over the last year, leading the DEA to establish an emergency schedule on April 12, 2013. UR-144 was present in all three varieties of "Skyscraper," while AM-2201 was present in two varieties. AM-2201 had a higher EC_{50} value at CB_1 than THC, which may account for the psychotic symptoms experienced by "Skyscraper" users. This study illustrates how cannabinoid receptor data can be combined with analytical results to better understand pharmacodynamic effects exhibited by synthetic cannabinoid users.

Keywords: Synthetic Cannabinoids, Cannabinoid Receptor, Bioassay

S06

Protein Adducts of Nitrogen Mustard Chemical Warfare Agents to Human Serum Albumin as Potential Biomarkers of Exposure

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Introduction: Covalent protein adducts are formed when electrophilic xenobiotics bind to nucleophilic amino acids in biological proteins. Literature has demonstrated that the three most important protein nucleophiles for xenobiotic adduction are cysteine (Cys), lysine (Lys), and histidine (His). The chemical warfare agents HN-2 and HN-3 are nitrogen mustard blister agents that can cause tissue blistering, ocular and respiratory damage when an individual has been exposed to these compounds. The use of protein adducts can, in theory, allow for detection of HN-2 or HN-3 exposure long after urinary metabolites and/or parent compounds have been excreted from the body. Previous *in vitro* work in this project has demonstrated the ability of HN-2 and HN-3 to bind to Cys, Lys, and His residues in model peptides, in addition to His residues on hemoglobin (Hb).

Objective: The objective of this current work was to identify adducts on the protein human serum albumin (HSA), specifically adducts formed on Lys and His residues, which have not been previously reported. These adducts can have the potential to be specific biomarkers of exposure to these dangerous compounds.

Method: Adduct formation was induced by incubating HSA for 3 h at 37°C with HN-2 and HN-3 at 50:1 molar excess. The protein was then digested with trypsin and the resulting peptides were separated and analyzed using UHPLC-MS/MS. Analysis was performed on an Agilent 6530 Quadrupole Time-Of-Flight (QTOF) Mass Spectrometer equipped with a 1290 Infinity UHPLC and a ZORBAX Eclipse Plus C-18 Rapid Res HD (10 mm x 2.1 mm, 1.8 μm particle size) column. Gradient elution of tryptic peptides with water and acetonitrile mobile phases containing 0.1% TFA was utilized. Mass Hunter BioConfirm Software (Agilent Technologies) was used to identify specific sites of adduction.

Results: HN-2 and HN-3 adduction occurred on specific Lys and His residues on HSA that have not previously been reported. Adduction was confirmed using replicate samples, exact mass MS, and MS/MS analysis. Identified adducts were determined to be stable at 37°C for the three week analysis period.

Conclusion: Previous research has demonstrated that HN-2 and HN-3 have the capability of adducting to highly reactive Cys residues on biological proteins such as HSA and Hb. However, due to the high reactivity of these residues and the considerable level of endogenous and exogenous electrophilic xenobiotics typically present, this site may be blocked and unavailable for adduction in many individuals. Therefore, it is beneficial to search for other specific biomarkers of exposure based on alternative protein nucleophiles such as Lys and His, which are generally more abundant in proteins. Future work will assess *in vitro* adduction of HN-2 and HN-3 in spiked whole blood samples as proof of concept for these adducts as potential exposure biomarkers.

Keywords: Chemical Warfare Agents, Biomarkers, Protein Adducts

S07

Para-Methoxymethamphetamine and Para-Methoxyamphetamine Concentrations in Clinical Specimens Submitted for Drug Testing

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Introduction: Para-methoxymethamphetamine (PMMA) and para-methoxyamphetamine (PMA) are hallucinogenic synthetic substituted amphetamines. In 2012, reports of PMMA and PMA poisoning were reported in Israel and Norway. This is a brief report of 5 cases from Alberta, Canada in which PMMA and PMA were detected in urine specimens submitted for clinical drug testing.

Objective: This report will provide the PMMA, PMA, methylenedioxymethamphetamine (MDMA), methylenedioxyamphetamine (MDA), methamphetamine (MAMP) and amphetamine (AMP) concentrations detected in the urine specimens of five (5) patients. Data from one patient in which two specimens were collected two hours apart will also be presented. Cross-reactivity studies using Siemens EMIT® Amphetamines and MDMA Assays and the Thermo Fisher CEDIA® Amphetamines/Ecstasy Assay were conducted to determine the ability of these immunoassays to detect PMMA and PMA.

Method: Qualitative analysis was performed using immunoassay (Siemens EMIT® II Plus Amphetamines and Ecstasy Assays; Thermo Fisher CEDIA® Amphetamines/Ecstasy Assay) followed by full scan GC/MS analysis using an Agilent 6890/5975. Quantitation of the amphetamines was performed using an Agilent 6890/5973 GC/MS in SIM mode.

Patient Data

Patient	PMMA (ng/mL)	PMA (ng/mL)	MDMA (ng/mL)	MDA (ng/mL)	MAMP (ng/mL)	AMP (ng/mL)	Other
1	106,000	3,100	11,900	300	ND	ND	cocaine, levamisole
2-A (13:15 h)	1,460	140	140	<LOQ	480	<LOQ	not analyzed by full scan GC/MS
2-B (15:15 h)	7,550	750	700	<LOQ	2,600	110	cocaine, cocaethylene, levamisole, phenacetin, ketamine, lidocaine
3	420	110	2,600	390	ND	ND	cocaine
4	25,900	2,290	34,900	1,920	380	<LOQ	no other drugs detected
5	6,330	590	2,720	200	ND	ND	cocaine, cocaethylene, levamisole, codeine, buproprion, quetiapine metabolite

LOQ = 50 ng/mL; ND = None Detected

Cross-Reactivity Data (at 500 ng/mL cut off concentrations)

Analyte	Siemens EMIT® II Plus Amphetamines Assay (ng/mL)	Siemens EMIT® II Plus Ecstasy Assay (ng/mL)	ThermoFisher CEDIA® Amphetamines/Ecstasy Assay (ng/mL)
PMMA	10,000 (neg) – 25,000 (pos)	2,500 (neg) – 5,000 (pos) (package insert = 9,000)	500 (neg) – 600 (pos) (CEDIA® cross-reactivity table = 250)
PMA	5,000 (neg) – 10,000 (pos)	10,000 (neg) – 25,000 (pos) (package insert = 22,000)	2,500 (neg) – 3,000 (pos) (CEDIA® cross-reactivity table = 4,200)

Results: The quantitative values demonstrate a wide variation with PMMA levels ranging from 420 – 106,000 ng/mL and PMA values ranging from 110 – 3,100 ng/mL. MDMA was detected in all samples containing PMMA and PMA. Other drugs of abuse and common prescription drugs were also detected.The ability of an immunoassay to detect PMMA and PMA will vary, with the ThermoFisher CEDIA® Amphetamines/MDMA immunoassay more sensitive to these drugs than either the Siemens EMIT Amphetamines Reagent or the Siemens EMIT® MDMA Reagent.

Conclusion: The data demonstrates the wide variability in PMMA and PMA concentrations found in specimens submitted for clinical urine drug testing. Laboratories need to understand the varying ability of immunoassays to detect these designer amphetamines and the implications for the population being tested.

Keywords: Para-Methoxymethamphetamine, Para-Methoxyamphetamine, Methylenedioxymethamphetamine

S08

Prevalence of Heroin Markers in Pain Management Patients

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Introduction: Recent reports have addressed heroin abuse in pain patients. However, the prevalence of heroin markers in this population has not been fully explored.

Objective: To characterize the prevalence of heroin-specific markers in urine for chronic pain patients, both in presence and absence of morphine.

Method: The study was approved by an Institutional Review Board. Approximately 920,000 urine specimens from chronic pain patients in 38 states were tested by liquid chromatography (LC) tandem mass spectrometry (MS/MS) for diacetylmorphine (DAM), 6-acetylmorphine (6AM), 6-acetylcodeine (6AC), codeine (COD), morphine (MOR), and norcodeine (NCOD). The limit of quantitation (LOQ) was 4 ng/mL for DAM, 6-AM and 6-AC, and 50 ng/mL for MOR and other opiates.

Results: A total of 2871 (0.31%) urine specimens were positive for one or more heroin-specific markers, with the following prevalence: DAM 1203 (41.9%), 6AM 2570 (89.5%), 6AC 1082 (37.7%). MOR was present in 2194 (76.4%) and absent (<LOQ) in 677 (23.6%) of the heroin-positive specimens. COD was present in 1218 (42.4%) specimens. In many cases, multiple heroin markers were present in a single specimen. Prevalence of combinations for specimens containing MOR were as follows: 6AM only 1140 (52.0%), DAM/6AM/6AC 710 (32.4%), 6AM/6AC 188 (8.6%), DAM/6AM 113 (5.2%), 6AC only 24 (1.1%), DAM only 13 (0.59%), DAM/6AC 6 (0.27%). Prevalence of combinations for specimens without MOR were as follows: 6AM only 217 (32.1%), DAM only 161 (23.8%), DAM/6AM 145 (21.4%), 6AC only 92 (13.6%), DAM/6AM/6AC 50 (7.4%), 6AM/6AC 7 (1.0%), DAM/6AC 5 (0.74%). Some, but not all, specimens were tested for benzoylecgonine (LOQ 20 ng/mL).

Characteristics of morphine positive and negative specimens are compared in the table below.

Parameter	Morphine Positive (N=2194)	Morphine Negative (N=677)
# DAM positives (%, Median, ng/mL)	842 (38.4%, 22.6)	361 (53.3%, 12.9)
# 6AM positives (%, Median, ng/mL)	2151 (98.0%, 194.8)	419 (61.9%, 14.2)
# 6AC positives (%, Median, ng/mL)	928 (42.3%, 16.3)	154 (22.7%, 5.9)
Prescribed Methadone	198 (9.0%)	74 (10.9%)
Prescribed Buprenorphine	160 (7.3%)	77 (11.4%)
Positive for Benzoylecgonine	442 (20.1%)	28 (4.1%)

Conclusion: A significant number of pain patients who provided urine specimens positive for heroin-specific markers exhibited an absence of morphine. While concentrations of DAM, 6AM, and 6AC tended to be lower for morphine-negative specimens, all three analytes appear to be useful biomarkers to determine evidence of heroin ingestion. Methadone and buprenorphine, which are used for the treatment of opioid dependence, were prescribed in a significant number of morphine-negative specimens. Despite sharing a common metabolic pathway with 6AM, benzoylecgonine was observed in some heroin positive/morphine negative specimens. The pathophysiologic explanation for finding heroin-specific markers in urine in the absence of morphine remains to be elucidated.

Keywords: Heroin, Pain Management, Urine Tests

S09

Analysis of Dietary Supplements with a Mammalian Cell-Based Androgen Receptor BioAssay and GC/MS

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Introduction: Under the Dietary Supplement Health and Education Act (DSHEA), products sold as dietary supplements are not regulated by the FDA. Supplement manufacturers are not required to provide proof of safety or effectiveness of their products. The supplement market has been inundated with new, designer compounds that allege to be anabolic or performance enhancing. These compounds are often difficult to detect with traditional chromatography/mass spectrometry methods because of minor structural modifications. There is rarely documented biological activity data associated with these compounds.

Objective: A mammalian cell-based human androgen receptor (hAR) bioassay that measures the relative anabolic bioactivity of compounds was developed. This bioassay was used to evaluate the anabolic activity of dietary supplements. The dietary supplement extracts were also analyzed by gas chromatogtaphy/mass spectrometry (GC/MS) to verify the presence or lack of purported performance enhancing compound(s).

Method: A 100mg aliquot of each supplement was dissolved in 1mL methanol, vortexed 30 seconds, rotated 10 minutes, and centrifuged 10 minutes at 1000xg. These sample extracts were used for the bioassay. A full length hAR plasmid and PB-ARE-2 plasmid were transfected into COS-7 cells using Lipofectamine 2000 CD™. After transfection, cells were treated with supplement extracts (in a dilution series: 1:1, 1:2, 1:5, 1:10, 1:25, 1:50, 1:100, 1:200, 1:500, 1:1000, 1:2000), testosterone standards (0.5-250ng/mL), or vehicle. After 24h sample treatment, cells were lysed and a Dual Luciferase Reporter Assay System® was used to determine luciferase activity. Luciferase activity was quantified using a VICTOR™ X4 Multilabel Plate Reader. The values [in response units (RUs)] obtained from measurement of firefly luciferase were normalized to values obtained from measurement of renilla luciferase in the same sample and expressed as fold induction of luciferase produced. The assays were averaged and SEM calculated. Data was analyzed with one-way ANOVA, p-value < 0.05 considered significant (GraphPad Prism 6). Supplement extracts were analyzed by GC/MS in scan mode.

Results: Three dietary supplements were chosen because they were expected to produce a negative response in the bioassay: 3Plenish, BCAA 3:1:2™, and Creatine Fuel®. As expected, these supplements did not activate the androgen receptor and produced no bioactivity. Several products were chosen for evaluation because they were suspected to contain performance enhancing compounds based on listed ingredients: Halodrol Liquigels™, Alpha1-T, Chlorodrol50™, 11-Oxo®, Formadex, and Charger™ Test. These products produced a non-negative response in the bioassay and were compared to a standard curve of testosterone activity. These six supplements produced activity similar to a 250ng/mL testosterone reference standard.

Conclusion: Designer compounds found in dietary supplements may not be detectable using traditional steroid analysis and there is likely no report of the anabolic activity of many of the compounds found in these products. 11-Oxo® (adrenosterone), for example, has been marketed as a cortisol suppressor but clearly activates the hAR as indicated by these experiments. It has been demonstrated that this assay may be used to indicate the presence of compounds that activate the hAR in dietary supplements directing further analysis by GC/MS to identify the active ingredient.

Keywords: Dietary Supplements, BioAssay, Bioactivity, Androgen Receptor

S10

Use of a Cannabinoid Receptor BioAssay to Determine the Potency (EC_{50})of Synthetic Cannabinoids and Metabolites

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Introduction: Cannabinoid receptor 1 and 2 (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 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. Most of these compounds are not well characterized and new compounds continue to be synthesized. K_i values are available in the literature for some of the parent synthetic cannabinoids. These K_i values were often determined from competitive receptor binding assays and are measurements of ligand affinity for the receptor; the K_i value is not necessarily indicative of the biological potency of the ligands. There has yet to be a comprehensive evaluation of the receptor-mediated biological activity or calculation of potency (as defined by the half maximal effective concentration, (EC_{50}) of parent compounds and/or metabolites of these compounds).

Objective: The potency (EC_{50}) of several synthetic cannabinoids and metabolites was determined using mammalian cell-based CB_1 and CB_2 bioassays and compared to reported K_i values.

Method: Analytical standards were obtained for synthetic cannabinoid parent compounds, metabolites, glucuronide-conjugated metabolites, THC, THC-COOH, and THC-11-OH. Standard curves were prepared and evaluated in an optimized CB_1 or CB_2 bioassay. Chinese hamster ovary (CHO) cells expressing either CB_1 or CB_2 were plated in 96-well ½ area plates and stimulated with forskolin and varying concentrations of the analytical standards. Changes in cAMP in the cells were measured with an optimized LANCE® Ultra cAMP Assay from Perkin Elmer. Each concentration of the dose-response curve was run in triplicate. Dose-response data were fit with a nonlinear regression model [log(dose) vs. response curve (three parameter)] to calculate the EC_{50} (GraphPad Prism 6).

Results: Forty analytical standards including UR-144, XLR-11, AKB-48, and several JWH compounds were evaluated in the CB_1 and CB_2 bioassays. Several synthetic cannabinoids such as HU-210, JWH-398, and AM-2201 have greater potency at both CB_1 and CB_2 than THC and THC metabolites. Many synthetic cannabinoid metabolites such as the JWH-018-N-(5-hydroxypentyl) metabolite and the UR-144 N-(4-hydroxypentyl) metabolite have substantial biological activity at CB_1 and/or CB_2; some with comparable potency to the parent compounds.

Conclusion: In addition to determining the potency of parent synthetic cannabinoids, it was demonstrated that many metabolites of synthetic cannabinoids are active at CB_1 and/or CB_2. Additionally, we have compared the calculated EC_{50} values to the reported K_i values.

Keywords: Synthetic Cannabinoids, Bioactivity, EC_{50}

S11

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

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Introduction: Arizona has a driving under the influence of drugs (DUID) administrative Per Se law. Arizona Revised Statute (ARS) 28-1381A(3) makes it unlawful for a person to drive or be in actual physical control of a vehicle while there is any drug as defined in section ARS 13-3401 or its metabolite in the person's body; which includes cannabis. As a result, Arizona has a relatively large number of DUID cases in which drivers are stopped, a drug recognition evaluation (DRE) is attempted or performed, a blood sample is obtained, and a cannabinoid or its metabolite is detected; this finding results in the charge of ARS 28-1381A(3). In some instances, the measured delta-9-tetrahydrocannabinol (THC) or THC metabolite is detected at very low concentrations.

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

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

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

Keywords: DUID, Driving, Cannabinoid, DRE, Blood, THC

S12

On Human Ethanol Pharmacokinetics: Time to Maximum Concentration and the Elimination Phase; Categorization of Profiles

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Introduction: Pharmacokinetic aspects of alcohol is frequently presented in legal applications for blood/breath alcohol concentrations at some time frame of interest: expert opinion evidence is provided from a subsequent analytical test result and/or from a consumption scenario. This study provides pharmacokinetic data on alcohol from forensic subjects to extend the foundation of literature support.

Objective: To present pharmacokinetic data on ethanol (alcohol) from testing 796 human subjects.

Method: Breath alcohol testing was conducted on 796 human subjects. Subjects were advised to consume a light snack prior to arrival in an alcohol-free state and were dosed with commercial alcohol-containing beverages specified in their forensic history. Breath alcohol concentrations were serially measured with an Intoxilyzer 5000 or Breathalyzer 900/900A instrument about every 15 minutes after dosing (drinking). Instrument calibration was verified using commercial alcohol aqueous solutions and gas mixtures.

Results: The median time for subjects to complete their last drink, with occasional prompting, was 21 minutes (range <1 to 128 minutes) with 11.2% of subjects completed by 10 minutes, 64.8% from 11 to 30 minutes, 21.9% from 31 to 60 minutes, and 2.0% from 61 to 75 minutes. The longest completion of 128 minutes was a method outlier from an exceptional large dose (360 mL of soju at 22% alcohol v/v). The median time for subjects to their highest alcohol measurement after drinking was 23 minutes (range: 3 to 109 minutes), with 72.2% of subjects by 30 minutes, 96.1% by 60 minutes, 99.5% by 90 minutes, and 100% within 120 minutes. The median time for subjects found in the elimination phase of alcohol from their system was 25 minutes (range 3 to 163 minutes) with 60.8% of subjects by 30 minutes, 88.7% by 60 minutes, 96.1% by 90 minutes, and 99.2% by 120 minutes. The longest time to elimination was 163 minutes for an otherwise non-distinct subject with their highest alcohol measurement at 15 minutes after drinking. Pharmacokinetic profiles were categorized: 65.6% of subjects had absorption followed immediately by elimination (linear), 16.3% had a peak followed by elimination, 10.9% had a plateau followed by elimination, 3.3% had a peak followed by a plateau and elimination, 2.0% had biphasic elimination (presumed from food) and 1.9% had a distinct time frame of increased variability in concentration after drinking.

Conclusion: This study contributes scientific support for a legal presumption time limit of two hours that is used in some jurisdictions for evidentiary breath and/or blood alcohol test results of motor vehicle operators.

Keywords: Ethanol, Pharmacokinetics, Breath

S13
Alprazolam: Alabama's Most Prevalent Drug in DUI/D Cases

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Introduction: Alprazolam (Xanax®), used to treat anxiety disorders and panic attacks, is the most frequently prescribed benzodiazepine and the tenth most commonly prescribed drug in the United States. The prescription drug misuse and abuse epidemic has led to an increase in driving cases involving alprazolam across the nation.

Objective: To investigate the prevalence of alprazolam in DUI/D cases in Alabama and characterize observations and behavior noted by Drug Recognition Experts (DREs).

Method: Blood samples from apprehended drivers suspected of driving under the influence of drugs from 11/2008-8/2012 were analyzed for alprazolam. Samples were screened by ELISA. Quantitation was performed by solid phase extraction and GC/MS (LOD/Q = 10 ng/mL) or liquid-liquid and LC/MS/MS (LOD/Q = 1 ng/mL). Twenty DRE evaluations involving alprazolam in blood were conducted from 5/2009 to 7/2012.

Results: We identified 714 alprazolam positive cases with an overall prevalence rate of 21%. 95% of alprazolam-positive cases were Caucasian with a mean age of 36 years-old. 78% of cases involved polydrug use and hydrocodone was the most common additional drug found (37%). The median concentration of alprazolam was 74 ng/mL. The range was 1.4 – 530 ng/mL. Geomapping illustrated pockets of high prevalence around major cities such as Birmingham, Mobile, and Montgomery.

Two DRE cases are presented below.

Case #1: A 33 year-old Caucasian female was involved in a single motor vehicle crash. The suspect displayed glassy, red eyes, droopy eyelids (ptosis), a flushed red face and slurred, thick-tongued speech (dysarthria). Her mood fluctuated between calm/cordial and hostile/abrasive. The subject displayed horizontal gaze nystagmus (HGN), lack of convergence, constricted pupils, and slow reaction to light. During the walk and turn (WAT), she fell out of starting position four times, missed heel to toe, stepped off the line, and took twelve steps. The suspect put her food down seven times, swayed while balancing, and used her arms for balance during the one leg stand (OLS). She admitted to taking four Klonopin® pills. Toxicology revealed alprazolam at 280 ng/mL and hydrocodone at 91 ng/mL.

Case #2: A 36 year-old Caucasian male was pulled over for traveling 79 mph in a 60 mph zone. The individual displayed slurred speech, unsteadiness on his feet, HGN, VGN, and hippus. He stepped off the line four times, could not maintain balance, and performed an incorrect turn during WAT. The subject swayed during the OLS and performed the alphabet, counting, and finger to nose tests poorly. He admitted to taking an "old" prescription of Xanax® and Lortab®, as well as consuming two Bud Light® beers. Toxicology revealed alprazolam at 120 ng/mL and ethanol at 0.05%.

Conclusion: Historically, marijuana has been the most prevalent drug in DUI/D cases in Alabama and remains the most prevalent drug in many states. For the first time, alprazolam has surpassed marijuana as the most frequently encountered drug (excluding ethanol) in driving cases. It is not surprising to see alprazolam and hydrocodone in combination since benzodiazepines are often taken by pain patients to improve sleep, relax musculature, and relieve anxiety that may be attributed to or exacerbate the sensation of pain.

DREs noted incoordination, stumbling, slow/slurred speech, and droopy eyelids. Apprehended drivers displayed poor driving, psychomotor impairment, and poor performance on SFSTs. Contribution from polydrug use should be considered. However, an emphasis should be placed on public awareness and education of law enforcement and the judicial system regarding the increase in DUI/D cases involving alprazolam.

Keywords: Alprazolam, DUID, Hydrocodone, DRE

S14

Recommendations for Toxicological Investigation of Drug Impaired Driving and Motor Vehicle Fatalities

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Introduction: Drug impaired driving is a significant traffic safety problem in the United States and around the world. Forensic toxicology laboratories involved in this type of casework have a wide variety of capabilities and resources, and operate under varying local policies, resulting in large variations in the types of drugs being tested for and the sensitivity of the testing employed. This creates inconsistencies and confusion within the criminal justice system while also preventing the collection of accurate and reliable epidemiological data. A consistent approach to the testing of the specimens in DUID investigations will represent a significant advancement in the effort to reduce drug impaired driving and improve public safety.

Objective: The objective of this presentation is to present a series of consensus recommendations developed to provide forensic toxicology laboratories with guidelines for a minimum standard for the analysis of drug impaired driving casework and to encourage implementation of those practices. These recommendations cover blood, urine, and oral fluid specimens; and outline the scope and sensitivity of testing to be applied to detect the drugs most frequently identified in drug impaired driving cases.

Method:A survey of 376 forensic toxicology laboratories was conducted to identify those performing DUID testing. The 123 identified laboratories were then surveyed regarding their demographics, analytical capabilities, and current resources. The laboratories were also questioned regarding their compliance with the 2007 recommendations[1] along with what barriers prevented them from adopting those practices. A total of 96 laboratories provided sufficient information to be included in the analysis of the survey data.

A subset of these laboratories was further surveyed for specific screening and confirmation cut-offs, along with drug prevalence data from their DUID casework. These laboratories where then invited to participate in a consensus meeting to review the 2007 recommendations, the survey data, the current literature, and to provide updated recommendations.

Results:A two tiered approach to testing was developed. Tier 1 compounds represent the most prevalent drugs in the U.S. driving population, and those for which there is the strongest evidence of impairment. Tier 1 compounds are all capable of being detected with available commercial immunoassays with standard cut-offs and confirmed using gas or liquid chromatographic instrumentation utilized in most DUID laboratories.

Compounds in Tier 2 are those recognized to be relevant to impaired driving investigations, but may be less frequently encountered, only of regional significance, and/or beyond the routine capability of many laboratories.

Conclusion: The goal of this presentation is to provide a framework in which impaired driving toxicology casework will be standardized both in DUID investigations and traffic fatality cases, and to encourage laboratories to adopt this standard approach. Implementation of Tier 1 testing will ensure that appropriate testing is being applied to DUID casework and will allow for more uniform data collection to better quantify the scope and nature of the DUID problem in the United States.

Keywords:DUID, Recommendations, Driving Impairment

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[1]Farrell, L.J., Kerrigan, S., Logan, B.K. (2007) Recommendations for toxicological investigation of drug impaired driving. Journal Forensic Sciences, 52, 1214-121.

S15
Case Management in a DUI Lab: Effect on Drugs Reported

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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 ethanol level. At the Palm Beach County Sheriff's Office (PBSO) Toxicology Lab there are two criteria that a case must meet before a BDS is conducted. First there must be a request. Second, if the case does not involve a fatality, the ethanol concentration must be less than 0.1 g/dL. If the case does involve a fatality then the requested BDS is peformed regardless of the ethanol concentration. It has been reported that by employing such a protocol, the number of drugs involved in DUI cases is vastly under reported.

Objective:A study was conducted to evaluate the magnitude of the under reporting 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.

Method:A BDS was performed on all DUI blood cases with sufficient volume submitted during a three month period, regardless of whether a BDS was requested by the officer, the ethanol level, or severity of the case. The three month period was from October 18, 2012 until January 18, 2013. The BDS consisted of a nine panel enzyme linked immunosorbent assay (ELISA) utilizing kits from Neogen (Amphetamine Ultra, Barbiturates, Benzodiazepines, Carisoprodol, Cocaine/Benzoylecgonine, Methamphetamine/MDMA, Opiates, Oxycodone/Oxymorphone, and THC) and a liquid/liquid extraction followed by analysis on gas chromatography/mass spectrometry (GC/MS) for determination of basic drugs not covered in the ELISA.

Results: In a three month period, 56 blood cases were submitted for toxicology analysis for antemortem DUI cases. Two samples had insufficient volume for a BDS and therefore were excluded from the study. Based on the case management protocol 38 of the 54 cases studied (70%) would not have received a BDS; all due to an ethanol level of greater than 0.1 g/dL. Sixteen of the 38 in the study were negative for drugs (42%). Of those cases with positive results, the drug results were considered to be significant for the purposes of this study if the ethanol level was below 0.2 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). Only 2 of the cases positive for drugs had results that were determined to be significant when evaluated in light of the corresponding ethanol level (5% of the 38 in the study group). Case 1 had an ethanol concentration of 0.124 g/dL and also contained alprazolam at 121 ng/mL. Case 2 contained ethanol at 0.155 g/dL as well as cocaine at less than 50 ng/mL, cocaethylene at less than 50 ng/mL, benzoylecgonine at 67 ng/mL, methadone at less than 100 ng/mL, diazepam at 78 ng/mL, nordiazepam at 167 ng/mL, oxazepam at less than 20 ng/mL, alprazolam at 77 ng/mL, and meprobamate at 5.3 ug/mL.

Conclusion: The decision to implement a protocol for limiting drug testing based on ethanol concentration is supported by the following: the known impairment associated with ethanol at higher concentrations, difficulty assigning a level of contributing impairment from drugs in the presence of high ethanol levels, and the likelihood that the drug results may be suppressed at trial. Although the results of this study support the assertion that such protocols may lead to under reporting drugs in antemortem DUI cases, these results definitively support that for the majority of cases the drugs detected are not significant, and do not warrant the significant increase in analysis required with blood drug analysis.

Keywords: DUI/DWI, Drugs, Case Management

S16

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

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Introduction: Ethanol is the most frequently identified compound in forensic toxicology. Although confirmation involving mass spectrometry is desirable, relatively few methods have been published to date. Other volatiles are commonly abused as inhalants. The methods used for identification of those inhalants are generally non-specific if analyzed concurrently with ethanol or require an additional analytical procedure that employs mass spectrometry.

Objective: A novel technique utilizing a capillary flow technology (CFT) splitter to simultaneously quantitate and confirm ethyl alcohol and identify inhalants by flame-ionization (FID) and mass spectrometric (MS) detection after headspace sampling and gas chromatographic separation is presented.

Method: The LOD and LOQ was determined by analyzing standards prepared in whole blood, urine, and aqueous matrices at successively lower ethanol concentrations down to 0.005 g/dL. The LOQ was determined as the lowest concentration that could be detected with an accuracy of ± 10% and a S/N of greater than 10:1. The LOD was determined as the lowest concentration detected with a S/N of greater than 3:1. Using 100 uL of sample, the limit of detection (LOD) and limit of quantitation (LOQ) were 0.005 and 0.010 g/dL, respectively for ethanol. The zero-order linear range ($r2 > 0.990$) was determined to span the concentrations of 0.010 to 1.000 g/dL. Within-run and between-run repeatability was evaluated by analyzing ten replicates of the 0.010 g/dL standard on three separate days by three different analysts and ten replicates of the 0.025, 0.080, and 0.300 g/dL standards on four separate days by four different analysts. The coefficient of variation (CV) of replicate analyses was less than 3.1%. Quantitative accuracy was within ± 8%, ± 6%, ± 3%, and ± 1.5% at concentrations of 0.010, 0.025, 0.080, and 0.300 g/dL, respectively. In addition, methanol, isopropanol, acetone, acetaldehyde, toluene, methyl ethyl ketone (MEK), isoamyl alcohol, isobutyl alcohol, n-butyl alcohol, 1,1-difluoroethane (DFE), 1,1,1-trifluoroethane (TFE), 1,1,1,2-tetrafluoroethane (Norflurane, HFC-134a), chloroethane, trichlorofluoromethane (Freon®-11), dichlorodifluoromethane (Freon®-12), dichlorofluoromethane (Freon®-21), chlorodifluoromethane (Freon®-22), and 1,2-dichlorotetrafluoroethane (Freon®-114) were validated for qualitative identification by this method. The validation for qualitative identification of volatiles other than ethanol included evaluation of matrix effects, sensitivity, carryover, specificity, repeatability and ruggedness / robustness. The validation for identification and quantitation of ethanol included evaluation of headspace oven thermostat time, thermostat stability, sensitivity, linearity, matrix effects, carryover, repeatability, drift/bias, specificity, reportable range, and a crossover case comparison.

Results: Since October of 2010, over 730 whole blood antemortem DUI or sexual assault case samples and 92 proficiency samples from the Florida Department of Law Enforcement Alcohol Testing Program (ATP) were analyzed for ethanol and other volatiles by this method. Ethanol was detected and quantified in 508 of those case samples (70%) and all of the proficiency samples. The duplicate positive results from the case samples typically agreed within 0.004 g/dL. All proficiency samples were determined to be satisfactory by ATP.
DFE was identified by this method in one urine and nine whole blood antemortem DUI case samples since June of 2010. Toluene was identified in one whole blood antemortem DUI case sample over this same time period.

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

Keywords: Ethanol, Volatiles, Inhalants, HS-GC-FID/MS

S17

Long-Term Stability of Ethanol in Blood Samples Collected from Suspected Drunk-Drivers

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Introduction: Alcohol (ethanol) misuse is common in the United Kingdom and drunk driving is known to cause approximately 25% of all road traffic deaths in Scotland. Therefore, it is important to ensure accurate and reliable analyses of samples from drivers suspected of driving under the influence of alcohol. Drivers providing a breath alcohol measurement over the prescribed limit (35µg/dL) are given the option to provide a self-submitted blood sample which is split and aliquots are independently analyzed. Blood samples must be collected in vials containing both a preservative and anticoagulant to ensure ethanol stability. Drivers are encouraged to store their blood sample in the refrigerator and submit for analysis within 1 week of collection; however, many samples are not submitted for analysis for a prolonged period of time and often storage conditions are unknown.

Objective: To investigate the stability of ethanol in samples collected from suspected drunk-drivers following storage at varying temperatures and after various lengths of time.

Method: A total of 412 blood samples from suspected drunk-drivers originally submitted to Forensic Medicine and Science for investigation were separated into two groups based on storage conditions and length of time in storage. Group A (N = 188) specimens were subjected to different storage conditions (room temperature, 4°C refrigerated, and -20°C frozen) over a period of 4-8 years. Group B (N = 224) specimens were refrigerated and then frozen following case conclusion; these samples were stored for 8-52 months. Analysis and re-analysis of all blood samples was carried out using an in-house validated method accredited to ISO/IEC 17025 and utilizing dual-column headspace gas chromatography with flame ionization detection (HS-GC-FID). Original analyses and repeat analyses were carried out in duplicate with the criteria for acceptability requiring a coefficient of variance of less than 2%.

Results: Ethanol loss from Group A samples averaged 33% (median 31%) following reanalysis after long-term storage at room temperature. Further storage of these samples for a prolonged period in the freezer resulted in average ethanol loss of 12% (median 3%). Group B samples, stored in the freezer following a short period in the refrigerator, demonstrated an average ethanol loss of 9% (median 6%) after reanalysis. In all 3 groups a small number of samples were observed to have lost 100% of the original ethanol concentration and in some cases a 100% gain.

Conclusion: Significant loss of ethanol was observed following long-term storage of blood samples at room temperature. Blood ethanol loss was reduced following long-term frozen storage. Blood samples stored in the refrigerator and then frozen had minimal loss of ethanol following long-term storage of between 8 -52 months. These findings confirm previous reports showing blood ethanol instability after long-term room temperature storage. These results also validate accuracy of blood ethanol analyses after refrigerated and frozen conditions up to 8 years in most cases.

Keywords: Ethanol Stability, Drunk Drivers

S18

Correlation of Alcohol in Oral Fluid with Breath Alcohol at Electronic Music Dance Events

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Introduction: Oral fluid has been used as a test matrix for the detection of alcohol and drugs at electronic music dance events that occur at clubs in the Bay Area. Alcohol has been shown to have saliva: blood ratio of 1.07 (Jones 1979), so estimates of blood alcohol concentration can be established by measuring alcohol content in oral fluid (OF). As well as the OF collection, individuals entering and leaving the events were asked to provide a breath alcohol (BrAC) test.

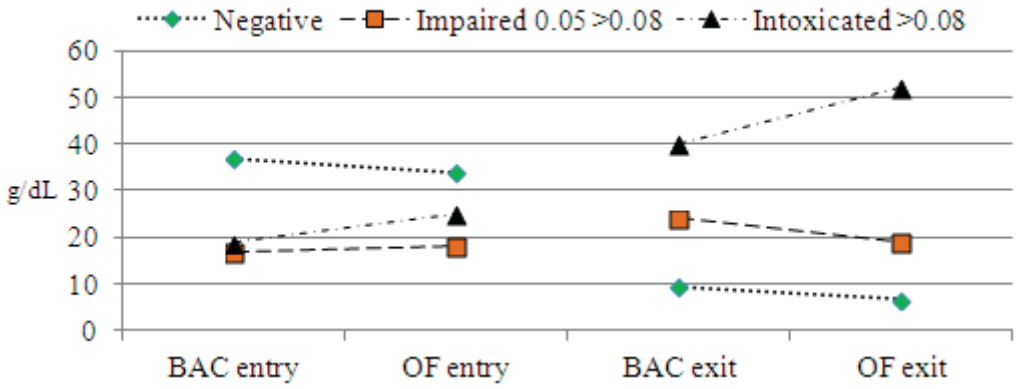
Objective: To determine the correlation between OF alcohol measurement collected with a Quantisal™ device and BrAC readings obtained using Intoxilizer 400PA breathalyzer units.

Method: Individuals entering and leaving clubs in the Bay Area volunteered to participate in the study. Not all participants gave both entry and exit specimens; only individuals who gave both an oral fluid and a BrAC result were included in this data set. Concentrations below 0.02g/dL were not considered. No special precautions were taken to avoid the presence of alcohol which was potentially present in the mouth.

Results: Oral fluid and breath alcohol were measured in 1366 people, of whom 1253 individuals provided both BrAC and OF specimens at entry and exit points. Demographically, gender was almost equally divided and the average age was 27 years. At entry 37% of subjects were negative (<0.02g/dL) using BrAC, 34% using OF; at exit 9.4% were negative by BrAC; 6.7% by OF.

At entry, 60.4% of females and 65.1% of males were positive (>0.02g/dL) in both matrices: concentration correlation $r^2 = 0.732$ and $r^2 = 0.661$ respectively. At exit 95.4% of females and 88.4% of males were positive in both matrices: $r^2 = 0.651$ and $r^2 = 0.657$ respectively.

When considering potential impairment (concentration range 0.05 – 0.08g/dL), the numbers from BrAC and OF were also similar (16% BrAC and 18% OF individuals identified at entry; 24% BrAC and 19% OF at exit). At entry 18% of the volunteers were over the legal driving limit of 0.08g/dL using BrAC; 25% using OF. At exit, as expected, BrAC identified 40% of subjects as >0.08g/dL; OF identified 52% as being >0.08g/dL.



Conclusion: Identification of alcohol levels was established using both biological matrices. The statistics between demographic groups were remarkably similar and the correlation between oral fluid alcohol content and BrAC was consistent. The collection of the oral fluid sample allows for the analysis of drugs in addition to alcohol, therefore the collection of only one matrix is necessary. Using oral fluid to determine alcohol content is viable, and reflective of BrAC concentrations.

Reference:

1. Jones AW. **(**1979**)** Inter- and intra-individual variations in the saliva/blood alcohol ratio during ethanol metabolism in man.*Clin Chem* 25(8): 1394 - 1398

Keywords: Alcohol, Oral Fluid, Breath Alcohol, Dance Clubs

S19
Disposition of Oxycodone and Hydrocodone in Oral Fluid

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Introduction: The use of oral fluid as a biological matrix for testing pharmaceutical products continues to expand in different areas including workplace, pain management, treatment and legal systems. Oxycodone (OC) and hydrocodone (HC) are the most frequently prescribed opioid medications in the United States, but there is limited information on the disposition of these drugs in oral fluid.

Objective: 1) Determine the time course of OC and HC and their respective metabolites in oral fluid following a single therapeutic dose in healthy, drug-free subjects; and 2) establish a database of parent drug and metabolites that might assist in interpretation of oral fluid test results.

Method: Two separate groups of subjects (n=12) were administered a single 20 mg OxyContin® tablet (extended release) or two 10 mg Norco tablets (immediate release). The studies were approved by an Institutional Review Board and informed consent was obtained. Neat oral fluid was collected by expectoration at timed intervals for 52 hrs. Specimens were frozen and shipped to the laboratory for analysis by LC-MS-MS (limit of quantitation = 1 ng/mL) for OC analytes [OC, oxymorphone (OM), noroxycodone (NOC)] and for HC analytes [HC, hydromorphone (HM), norhydrocodone (NHC) and dihydrocodeine (DHC)].

Results: The relative abundance of OC and HC analytes detected were as follows: OC>NOC>OM and HC>>NHC>DHC. The mean Cmax (ng/mL) (N, mean, SEM, range) were as follows: OC (12, 132.7, 15.4, 49.2-218.7); NOC (12, 18.7, 1.6, 10.3-31.8); OM (7, 1.6, 0.2, 1.2-2.4); HC (12, 207.7, 42.0, 61.7-625.6); NHC (12, 12.8, 2.4, 3.6-27.0); and DHC (12, 6.4, 1.2, 2.6-18.2). The mean Tmax (hrs) (N, mean, SEM, range) were as follows: OC (12, 3.3, 0.4, 2.0-6.0); NOC (12, 5.1, 0.6, 2.5-8.0); OM (7, 3.7, 0.3, 2.0-4.0); HC (12, 1.4, 0.3, 0.3-4.0); NHC (12, 2.6, 0.6, 1.0-8.0); and DHC (12, 4.5, 0.9, 1.5-12.0). The detection times (hrs, time to last positive) at 1 ng/mL cutoff concentration (N, mean, SEM, range) were as follows: OC (12, 34.3, 1.8, 28.0-54.0); and HC (12, 29.8, 2.6, 14.0-48.0).

Conclusion: Both OC and HC appeared rapidly (15-30 minutes) in oral fluid following dosing. Because OC was an extended release preparation, entry into oral fluid was slower and concentrations remained elevated longer relative to HC. Only trace amounts of O-demethylated metabolite (OM) were detected and no HM was detected. The N-demethylated metabolites were present at similar times as the parent drug but were generally in lower concentrations. This body of data should facilitate the continued development of oral fluid for detection of prescription opiates and assist in the interpretation of test results.

Keywords: Oral Fluid, Hydrocodone, Oxycodone, Metabolites

S20

Simultaneous Analysis of 19 Synthetic Cannabinoids and Their Contribution to Overall Positivity in Oral Fluid Samples

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Introduction: The number of synthetic cannabinoids available on the market has grown tremendously in the last few years. Regulatory agencies as well as the drug testing industry are facing challenges because new chemicals are so quickly introduced to circumvent current regulations. In July 2012, the United States DEA banned 5 classes of synthetic cannabinoids and 15 specific chemicals. Redwood Toxicology has been testing for JWH-018, JWH-073, JWH-081, JWH-210, JWH-250, RCS-4 and AM-2201 in oral fluid samples and noted a significant decrease in positivity following this ban. The existing analytical method was updated to include the newly regulated drugs: AKB-48, JWH-019, JWH-122, JWH-200, JWH-203, JWH-398, AM-694, MAM-2201, RCS-8, UR-144, XLR-11 and one still legal drug: AM-1248. The fully validated and updated method was applied to nearly 1000 authentic oral fluid specimens. The results from the validation and clinical specimens will be discussed.

Objective: To develop and validate a method for simultaneous analysis of 19 regulated and unregulated synthetic cannabinoids in oral fluid samples and to study the concentrations and contributions to the overall positivity.

Method: Quantisal collection devices were used, applying a 1:4 dilution of oral fluid in the device. Method validation studies included extraction efficiency, accuracy, imprecision, LOD/LOQ, ULOL,carryover, matrix effect, stability and interference from 99 related and non-related drugs. Calibration solutions were spiked usingdrugs and their deuterated analogues (when available) from Cayman Chemicals. Sample preparation involved the addition of 100 µl IS (in DMF) to 100 µl of specimen and centrifugation prior to removing an aliquot for LC/MS/MS analysis. LC/MS/MS parameters were as previously reported (SOFT 2012 abstract S37). The method was applied to 977 authentic oral fluid specimens with a cut-off of 0.25 ng/mL.

Results: Extraction efficiency from the device was > 50% for all analytes except AM-1248. The LOD and LOQ for all analytes were 0.1 and 0.25 ng/mL respectively. ULOL was 25 ng/mL or greater, giving an $R \geq 0.99$ and accuracies between 80-120% for all analytes. Inter and Intra-day imprecision was below 7.5%. No carry over was identified for any of the compounds up to 250 ng/mL. The %CV of the normalized matrix factors for all analytes was ≤13.4% for all analytes and was well within the acceptable criteria of <15%. No interference was noticed from any of the spiked drugs (1000ng/mL) in the negative and LOQ specimens. Prepared specimens were stable for 48hrs in the instrument autosampler (15^0C).

Of the 977 specimens tested, 82 (8.4%) were positive for one or more drug(s). XLR-11 was the most common analyte, detected in 73% of the positive specimens either alone (56%) or in combination with other drugs (17%). UR-144 was the second most prevalent (18%) of the positives followed by AM-2201(13%), AM-694 (11%), JWH-122 (5%), JWH-210 (5%), JWH-018 (4%) and MAM-2201 (4%).

Conclusion: Simultaneous analysis of 19 synthetic cannabinoids was successfully implemented in our laboratory**.** A total of 13 different types of SC were detected in routine oral fluid testing post ban implemented in July 2012. Oral fluid, once again, proves to be an ideal matrix to test emerging synthetic cannabinoids resulting from new regulations.

Keywords: Synthetic Cannabinoids, Oral Fluid, LC/MS/MS

S21

Monitoring Oral Fluid for Pyrolysis Products of XLR-11 and UR-144 as an Indication of XLR-11 and UR-144 Ingestion

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Introduction: XLR-11 and UR-144 are currently the most popular synthetic cannabinoids (SC) that the United States DEA proposes to place under emergency control. These tetramethylcyclopropyl (TMCP) analogs of previous generation SC appeared following a ban in June 2012 that placed SC in Schedule 1 category based on their structural classification. Redwood Toxicology Laboratory analyzes oral fluid specimens from court ordered programs for 19 SC including XLR-11 and UR-144. With an overall positivity of about 10% for SC, XLR-11 and UR-144 are detected in 72% and 18% of the positive specimens respectively. In all of the XLR-11 and/or UR-144 positives, unidentified peaks with the same molecular mass and similar mass fragments were noted. These peaks were later identified as the pyrolysis products of XLR-11 and UR-144 formed by opening of the TMCP ring due to thermal degradation during smoking.

Objective: Sixty oral fluids positive for XLR-11 and/or UR-144 parent drugs were re-analyzed to evaluate the concentrations of the pyrolysis products with respect to the parent drugs and to study the suitability of monitoring both parent and pyrolysis products for indicating exposure.

Method: Oral fluids were collected using Quantisal device resulting in a 1:4 dilution. Standard reference material for XLR-11, UR-144 and their pyrolysis products were obtained from Cayman Chemicals. Extensive extraction efficiency, matrix effect and specificity studies were carried out during method validation. LOD and LOQ for all analytes were 0.1 and 0.25ng/mL respectively. 100µl of sample was removed from the collection tube, diluted with dimethylformamide and centrifuged. 20µL was then subjected to LC/MS/MS analysis in positive MRM mode with ESI. Separation was performed on a biphenyl, 5µ column with a flow rate of 0.5 mL/minute. Mobile phases were 0.1% formic acid with 2mM ammonium formate and 0.1% formic acid with 2mM ammonium formate in acetonitrile. The gradient started at 10% organic and increased to 90% in 8 minutes before returning to original conditions. Method was applied to 60 authentic oral fluid specimens with an administrative cut-off of 0.25ng/mL.

Results: Parent XLR-11 was detected in 93% of the specimens either alone (78%) or in combination with UR-144 (21%). Parent UR-144 alone was detected in 4 specimens (6.6%). XLR-11 and UR-144 thermal degradation products were detected in all XLR-11 and UR-144 positives respectively. The ratio of the degradant /parent drug was >1 in 70% of the XLR-11 positives. Similar trend was noted for the UR-144 degradant in the UR-144 positive specimens. 10% of the total specimens re-tested had parent drug concentrations below LOQ (0.25ng/mL) but above LOD (0.1ng/mL) and degradant concentrations well above the cut-off (0.25ng/mL) and hence would be considered negative if tested only for the parent drugs.

Conclusion: Monitoring oral fluid for the pyrolysis products of XLR-11 and UR-144 in addition to the parent drug is recommended to avoid false negative results. The presence of only XLR-11 and/or UR-144 degradant may be indicative of exposure via smoking. Further research on the application of the pyrolysis products of XLR-11 and UR-144 for oral fluid and their metabolites for urine testing is needed.

Keywords: Synthetic Cannabinoids, Oral Fluid,XLR-11, UR-144, Pyrolysis

S22

Oxycodone and Hydrocodone: Kinetic Relationships of Whole Blood to Oral Fluid

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Introduction: The primary mechanism for transfer of drugs and metabolites from blood to oral fluid is passive diffusion. Factors influencing the diffusion of drugs and metabolites into oral fluid include specimen acidity, drug pKa, drug-protein binding, metabolic rates, and elimination rates. Understanding the kinetic relationship of drugs/metabolites in blood to oral fluid can facilitate the interpretation of oral fluid tests.

Objective: Characterize the kinetic relationship of oxycodone (OC) and hydrocodone (HC), and their associated metabolites in whole blood to oral fluid specimens collected at near simultaneous times.

Method: Two separate groups of subjects (n=12) were administered a single 20 mg OxyContin® tablet (extended release) or two 10 mg Norco tablets (immediate release). The studies were approved by an Institutional Review Board and informed consent was obtained. Whole blood and neat oral fluid (expectoration) specimens were collected at timed intervals for 52 hrs. Specimens were frozen and shipped to the laboratory for analysis by LC-MS-MS (limit of quantitation; blood, 5 ng/mL, oral fluid, 1 ng/mL) for all analytes [OC, oxymorphone, noroxycodone (NOC), HC, hydromorphone, norhydrocodone (NHC) and dihydrocodeine].

Results: Following either OC or HC administration, parent drug appeared within 15-30 minutes in whole blood and oral fluid. The appearance and disappearance of parent drugs and related normetabolites followed a similar time course in both matrices. Pharmacokinetic analysis (non-compartmental, model independent) estimates of terminal half-life ($T_{1/2}$) in oral fluid were closely related to those of whole blood (see table). Generally, oral fluid drug/metabolite concentrations correlated well with whole blood. As expected, parent drug concentrations in oral fluid were several-fold higher than in whole blood as demonstrated by oral fluid/blood ratios. In contrast, normetabolite concentrations tended to be similar in both matrices.

Kinetic Parameter	OC	NOC	HC	NHC
Oral fluid $T_{1/2}$, hr (N)	4.6 (12)	8.3 (12)	4.4 (12)	6.2 (9)
Blood $T_{1/2}$ (N)	5.6 (11)	11.8 (11)	4.5 (12)	7.7 (11)
Correlation, r, oral fluid:blood (N)	0.719 (12)	0.651 (12)	0.733 (12)	0.423 (11)
Oral fluid/blood ratio (N)	5.4 (12)	1.0 (12)	3.2 (12)	0.7 (11)

Conclusion: Although passive diffusion of drug from blood to oral fluid is subject to numerous influences that impart variability, concentrations of analytes in both matrices were closely correlated. These data demonstrate the ultra-rapid transfer of OC and HC from blood to oral fluid and the concurrent enhancement of oral fluid concentration that occurs during exchange. In conclusion, oral fluid tests for OC and HC are valid methods for detection of recent use of these drugs.

Keywords: Oral Fluid, Blood, Hydrocodone, Oxycodone, Kinetic Relationships

S23

Phase I and II Cannabinoid Disposition in Blood of Chronic, Frequent and Occasional Smokers Following Controlled Smoked Cannabis

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Introduction: Δ^9-tetrahydrocannabinol (THC) is detected in chronic, frequent smokers' blood for at least 30 days of sustained abstinence. Markers of recent cannabis smoking in blood are needed for correlation with acute impairment. THC-glucuronide was suggested as a marker, but was only evaluated in chronic smokers.

Objective: Quantify THC, 11-hydroxy-THC (11-OH-THC), 11-nor-9-carboxy-THC (THCCOOH), cannabidiol (CBD), cannabinol (CBN), THC-glucuronide, and THCCOOH-glucuronide in chronic, frequent (≥4x/week) and occasional (<2x/week) smokers' blood following controlled smoked cannabis.

Method: 14 chronic, frequent and 11 occasional cannabis smokers (18M, 7F) provided written informed consent for this IRB-approved study. Cannabinoids were quantified by LCMSMS from blood collected in sodium heparin tubes at study admission (19h before), 1h before and up to 15 times from 0.5 to 30h after *ad libitum* smoking of one cannabis (6.8% THC) cigarette. Limits of quantification (LOQ) were 1 µg/L for THC, 11-OH-THC, THCCOOH, CBD, and CBN; 0.5 µg/L for THC-glucuronide; and 5 µg/L for THCCOOH-glucuronide. Mann Whitney exact test evaluated group differences (significance at $p<0.05$).

Results: At baseline (-1h), THC, THCCOOH, and THCCOOH-glucuronide were detected in all, and 11-OH-THC in 43%, of chronic, frequent smokers' samples. In occasional smokers, only THCCOOH was detected in 27.3% of baseline blood samples. Within-group cannabinoid concentrations were highly variable. Median THC, 11-OH-THC, THCCOOH, and THCCOOH-glucuronide were significantly higher in chronic, frequent smokers at all time points, except for 11-OH-THC at 26, 28, and 30h post-dose. For CBD, CBN, and THC-glucuronide, only CBN at 0.5 h was significantly higher in frequent smokers.

Table 1: Positivity rate, maximal concentration and last detection time after smoking one 6.8% THC cannabis cigarette

Analyte	Parameter	Chronic Frequent Smoker	Occasional Smoker	p
THC	% Positive Participants	100	100	-
	Post-Dose C_{max} (µg/L)	34.4 (16.5-49.5)	12.1 (4.1-40.3)	0.007
	t_{last} (h)	>30 (24.0->30)	4.0 (1.0-6.0)	0.000
11-OH-THC	% Positive Participants	100	100	-
	Post-Dose C_{max} (µg/L)	6.2 (2.2-3.4)	2.9 (1.6-7.9)	0.003
	t_{last} (h)	12.0 (3.1->30)	3.0 (1.0-5.0)	0.000
THCCOOH	% Positive Participants	100	100	-
	Post-Dose C_{max} (µg/L)	48.7 (31.9-119)	10.4 (6.5-27.4)	0.000
	t_{last} (h)	>30	>30	N/A
CBD	% Positive Participants	15.4	0	-
	Post-Dose C_{max} (µg/L)	0 (0-1.5)	0	0.482
	t_{last} (h)	0 (0-0.5)	0	0.482
CBN	% Positive Participants	85	36.4	-
	Post-Dose C_{max} (µg/L)	2.2 (0-3.1)	0 (0-2.8)	0.048
	t_{last} (h)	0.6 (0-2.1)	0 (0-1.1)	0.046
THC-glucuronide	% Positive Participants	15.4	9.1	-
	Post-Dose C_{max} (µg/L)	0 (0-1.1)	0 (0-0.6)	0.717
	t_{last} (h)	0 (0-0.5)	0 (0-0.6)	1.000
THCCOOH-glucuronide	% Positive Participants	100	90.9	-
	Post-Dose C_{max} (µg/L)	74.3 (40.4-218)	16.2 (0-83.4)	0.000
	t_{last} (h)	>30	27.0 (0->30)	0.003

Conclusion: Higher concentrations were documented in chronic, frequent smokers for THC, 11-OH-THC, THCCOOH, and THCCOOH-glucuronide due to the high body burden developed over time. When present, CBD, CBN, and THC-glucuronide were detected at low concentrations and only for <2h. Presence of these analytes indicates recent use, but their absence does not exclude it. Smoking topography and enzyme polymorphisms may explain inter-individual variability.

Keywords: Cannabinoids, Glucuronides, Recent Use, Blood, Marijuana

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S24

Quantification of Synthetic Cannabinoids in Urine by Liquid Chromatography Tandem Mass Spectrometry

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Introduction: Synthetic cannabinoids are a large emerging drug class prevalent in forensic and workplace cases. Clandestine laboratories constantly produce new formulations to circumvent legislative efforts complicating toxicological analysis.

Objective: To develop and validate a liquid chromatography tandem mass spectrometric (LC/MS/MS) method for quantifying JWH-018, JWH-019, JWH-073, JWH-081, JWH-122, JWH-200, JWH-210, JWH-250, JWH-398, RCS-4, AM-2201, MAM-2201, UR-144, CP 47497-C7, CP 47497-C8 and their metabolites, and JWH-203, AM-694, RCS-8, XLR-11 and HU-210 parent compounds in urine.

Method: 4000 Units beta-glucuronidase was added to 0.2 mL human urine diluted with 0.3 mL 400 mM ammonium acetate pH 4.0 buffer prior to 2 h hydrolysis at 55˚C. Specimens were clarified by centrifugation after adding 0.4 mL acetonitrile. After transferring supernatant onto 1 mL Biotage SLE+ columns, analytes were eluted with 6 mL ethyl acetate and dried under nitrogen. Specimens were reconstituted in 150 µL mobile phase consisting of 50% A (0.01% formic acid in water) and 50% B (0.01% formic acid in 50:50 methanol: acetonitrile). 4 and 25 µL injections were performed to acquire data in positive and negative ionization modes, respectively. The LC/MS/MS instrument consisted of a Shimadzu UFLCxr system and an ABSciex 5500 Qtrap mass spectrometer with an electrospray source. Gradient chromatographic separation was achieved utilizing a Restek Ultra Biphenyl HPLC column (100 x 2.1 mm; 3 µm particle size) with a 0.5 mL/min flow rate and an overall run time of 19.5 and 11.4 min for positive and negative mode methods, respectively. Quantification was conducted in MRM mode with CP 47497 compounds and HU-210 ionized via negative polarity; all other analytes were acquired in positive mode. Quantifier and qualifier MRM transitions were monitored for each analyte and internal standard. Commercially available deuterated internal standards were utilized when available; deuterated analytes with similar functional groups and nearest retention time were utilized when matched deuterated internal standards were unavailable.

Results: Lower limits of linearity were 0.1-1.0 µg/L and upper limits of linearity were 50-100 µg/L (r^2> 0.994). Validation parameters were evaluated at three concentrations spanning linear dynamic ranges. Inter-day analytical recovery (bias) and imprecision (N=20) were 88.3-112.2% of target and 4.3-13.5% relative standard deviation, respectively. Extraction efficiencies (N=10) were 44-110%. Matrix effects (N=10) ranged from -28 to 52, and -34 to -73% for positive and negative modes, respectively. Analyte stability was assessed (N=3) after 16 h at room temperature, 72 h at 4˚C, and three freeze-thaw cycles. All analytes were stable at 4˚C for 72 h. All metabolites and JWH-200, CP 47497-C7, CP 47497-C8 and HU-210 were stable for 16 h at room temperature and after three freeze-thaw cycles; all other parent analyte concentrations decreased >20% after 16 h at room temperature and after three freeze-thaw cycles.

Conclusion: We present a novel analytical method for simultaneously quantifying 15 synthetic cannabinoids and metabolites and 5 additional parent compounds in urine from forensic and workplace cases. Instability could partially explain lack of parent analyte identification in urine.

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

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

S25

Analysis of Smoke Condensate from Herbal Cigarettes Laced with Fluorinated Synthetic Cannabinoids

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Introduction: Significant adverse events and emergency-room reports have been associated with synthetic cannabinoid-containing herbal product exposure, including acute kidney injury and psychiatric disturbance. However, it is not clear whether adverse effects are due to exposure to the herbal products or the synthetic cannabinoids used as adulterants, or alternatively involve synthetic impurities or pyrolytic products. Therefore, additional information regarding the chemical composition of these herbal products, as well as the chemical composition and yield(s) in smoke, is vital to understanding the relationship between chemical exposures and pharmacological and physiological consequences.

Objective: The aim of this work was to determine the biological exposures that occur during the smoking of synthetic cannabinoids in herbal formulations through the characterization of the chemical composition of mainstream and side-stream smoke condensates.

Method: Herbal cigarettes were prepared in triplicate from marshmallow leaf herbal material laced with one of a series of fluorinated synthetic cannabinoids and their non-fluorinated analogs: AM-2201, JWH-018, XLR-11, UR-144, 5-fluoro-PB-22, or PB-22. The triplicate cigarettes were smoked using a Borgwaldt KC smoking machine. Mainstream and side-stream smoke condensates were collected and remaining un-smoked remnants were recovered and extracted. All samples were analyzed by LC-MS using a Waters Synapt G2 Q-TOF high-resolution mass spectrometer and GC-MS using an Agilent 7001B triple quadrupole system. Smoke from control cigarettes containing only marshmallow leaf was also collected. Appropriate reference standards were analyzed to confirm identity and determine recovery.

Results: In herbal cigarettes containing JWH-018 a significant proportion of this synthetic cannabinoid was volatilized and delivered intake in the mainstream fraction, and was also detected in the unburned remnant and side-stream smoke. Only one pyrolytic degradant was detected, which was determined to be JWH-022, indicating that the combustion process desaturated the alkyl chain in the terminal position leading to the formation of an alkene. When the 5'-position of the alkyl chain of JWH-018 is fluorinated as in AM-2201, the same alkene containing degradation product (JWH-022) was observed. The loss of fluorine to form JWH-018 was also observed as a minor combustion product. The yields of AM-2201 in smoke were variable, but a significant portion (~50%) appeared to be transferred in mainstream smoke intact. The tentative identification of these compounds by mass spectrometry was confirmed by comparison of retention times and spectra obtained with JWH-022 and JWH-018 reference standards. For cigarettes laced with UR-144, *almost complete* conversion of UR-144 to a cyclopropyl ring-opened form was observed in the smoke condensates and cigarette remnant. This degradant has been previously described and is observed to occur over time in the bulk drug substance and in solution. XLR-11 also underwent rapid and almost complete thermal conversion to its ring opened form, lost fluorine to become UR-144, and ultimately converted to the alkene-containing ring-opened form of UR-144. Preliminary results with 5-fluoro-PB-22 and PB-22 also suggest significant thermal conversion occurs with these ester-containing synthetics.

Conclusion: Users experience significant exposure to intact parent compound and combustion products when synthetic cannabinoids are smoked. In some products, it appears that individuals are exposed primarily to combustion products. These products can be components that have an altered affinity to the CB1 and CB2 receptor, or entities of unknown pharmacology and toxicology.

Keywords: XLR-11, UR-144, PB-22, 5-Fluoro-PB-22, Spice, Mass Spectrometry, Cannabinoid

S26
Unusual Findings Compounded in a Pediatric Fatality

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Introduction: In early February 2012, the sudden unexpected death of an otherwise healthy six month old male was reported to the Los Angeles County Department of Coroner. Paramedics initiated resuscitation efforts and transported a cyanotic and asystole infant to the hospital, whom despite medical intervention was declared dead shortly after arrival. During the hospital code the baby showed a hemoglobin level of 8.8 gm%, blood glucose of 145 mg/dL, and a rectal temperature of 96.8 degrees Fahrenheit. The scene investigation was unremarkable and interviews with the family directed the investigation towards co-sleeping. Findings at autopsy were remarkable for severe pulmonary edema/congestion. The case was pended for histology and toxicology.

Objective: To present a tissue distribution of lethal drug levels in a pediatric death, as well as bring awareness to the forensic toxicology community on compounding pharmacies, off label drug use, and potential routes of administration.

Method: Toxicology analysis was performed in the heart blood, liver, and gastric contents. Barbiturates, Cocaine, Methamphetamine, Opiates, Phencyclidine, Marijuana, Acetaminophen, and Salicylates were screened via an enzyme linked Immunosorbent assay (ELISA). Volatiles analysis was performed on a Headspace Gas Chromatograph/Flame Ionization Detector (GC/FID). Basic drugs were analyzed utilizing a basic liquid/liquid extraction with qualification and quantification performed on a GC/Nitrogen Phosphorous Detector (GC/NPD) in combination with a GC/Mass Spectrometry Detector (GC/MSD).

Results: Toxicology tests for volatiles and ELISA drugs were negative. The following table represents drugs detected in the basic drug extraction.

	Heart Blood [ug/mL]	Liver [ug/g]	Gastric Contents [mg]
Amitriptlyine	0.59	5.3	0.28
Nortriptyline	0.11	1.3	---
Tramadol	10	11	0.80
Nortramadol	0.61	1.0	---
Dextromethorphan	1.0	3.6	0.33

Conclusion: The cause of death was determined to be multiple drug intoxication, however further investigation was required to determine the manner of death. Local law enforcement was notified of the unusual findings in the infant, which prompted a search of the residence. The search returned multiple prescription medications, as well as several compounding ointments including one 30g tube containing 4% Amitriptyline, 20% Tramadol, and 10% Dextromethorphan. The drugs detected in the infant can most likely be attributed to the compounding ointment, however little data exists on the efficacy, safety, and/or drug levels associated with the use of compounded preparations. Typically, compounding pharmacies fabricate drug formulations customized for the individual patient in order to facilitate administration and/or alter the ingredient composition per prescription. Compounding pharmacies are not regulated by the Federal Drug Administration and preparations account for only 1-5 % of filled prescriptions in the United States. Additional evidence recovered from the residence consisted of pediatric paraphernalia (baby bottles, pacifiers, teething rings, etc.). A simple methanol rinse of these items revealed one bottle in which amitriptyline and tramadol were identified. This finding, in combination with the drug levels in the gastric contents, suggests the route of administration may have been oral rather than topical. The manner of death was ruled a homicide.

Keywords: Pediatric Fatality, Compounding Pharmacy, Amitriptyline, Tramadol, Dextromethorphan

S27
Designer Drug Trends in Miami: Postmortem Case Reviews

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Introduction: The abuse of designer drugs, such as cathinone derivatives, is on the rise and severe intoxications and fatalities are not uncommon. The Miami-Dade County Medical Examiner Department has detected several designer drugs in dozens of post-mortem cases over the last couple of years. It is important to establish trends in abuse so that pathologists, toxicologists, first responders, investigators, and law enforcement can be well-informed on how to identify subjects who may have used such drugs.

Objective: The objective of this case study is to identify the compounds that are being detected in our cases as well as to establish trends regarding age, sex, race, cause of death, and behavior of the typical user of cathinone derivatives.

Method: Routine toxicological analyses were performed on post-mortem specimens, or ante-mortem specimens when available. All cases received a volatile analysis by GC-FID. Urine specimens were analyzed by EMIT and then subjected to liquid-liquid extraction for GC-MS analysis. Blood specimens were analyzed by ELISA and then subjected to solid-phase extraction for analysis by dual-column GC-FID, dual-column GC-TSD, and GC-Ion Trap/MS. The cathinone derivatives were successfully identified in our routine screening process by GC/TSD and GC/MS, and were further confirmed based on comparison to relative retention times and mass spectra of certified known reference standards.

Results: Since October 2011, our laboratory has detected designer drugs in 24 cases. Decedents, all males, ranged in age from 18-60 (mean 28). The most commonly detected compound has been methylone, followed by MDPV. Butylone and 4-MEC have been detected in one case each. Many cases did not involve alcohol or other drugs. In total, there have been 4 suicides (2 murder-suicides), 10 homicides, and 10 accidental deaths. Amongst the accidents, 3 were motorcycle crashes and 7 were overdose cases. Two of the deaths that were pending toxicology also involved huffing agents. Of the cases where specific drug names were indicated, “mollies” or Ecstasy were mentioned but not “bath salts”, indicating unintentional ingestion of these compounds. The homicide victims were all black males, age 18-31. The motorcycle victims were all white males, age 23-34. The decedents in the overdose cases were all white males, age 21-35. Other compounds similar to cathinone derivatives that were detected include: MDMA, 5-MeO-DiPT, BZP, TFMPP, MDA, phentermine, methamphetamine, amphetamine, fluoroamphetamine, and methiopropamine – several cases involving very complex combinations. Other drugs of abuse detected include: ethanol, cocaine, benzodiazepines, and analgesics. The ER reports and eye-witness accounts of the decedents who over-dosed on these drugs indicated the stereotypical signs and symptoms associated with bath salt use: aggression, paranoia, delusions, hyperthermia, serotonin-syndrome, cerebral edema, stroke, seizure, multiple-organ failure, disseminated intravascular coagulation, and ultimately death.

Conclusion: It is important to categorize trends, behaviors, drug terminology, and signs/symptoms related to bath salt use so that forensic professionals can identify the presence of these types of drugs. The abuse of such compounds is not uncommon in Miami, despite strict regulations. Users may be ingesting these analytes unknowingly in the form of “mollies” or Ecstasy. In addition, findings of these types of drugs may indicate behavior or impairment in the case of suicides, homicides, or motor-vehicle accidents.

Keywords: Designer Drugs, Postmortem, Bath Salts, Ecstasy

S28

Further Studies on Incorporation of Synthetic Cannabinoids in Different Organs and Toxicological Analysis

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Introduction: Synthetic cannabinoids are still an issue in forensic toxicology. Many data are already published regarding metabolism and detection in body fluids and hairs, but very little is known about their persistence in body tissues.

Objective: The aim of the present work was to investigate in which body samples and in which amounts the synthetic cannabinoids JWH-210 ((4-ethylnaphthalen-1-yl)(1-pentyl-1*H*-indol-3-yl)methanone)) and JWH-122 ((4-methylnaphthalen-1-yl)(1-pentyl-1*H*-indol-3-yl)methanone)) can be detected two, four, and six weeks after a single administration to rats.

Method: Extracts of herbal mixtures containing JWH-210 or JWH-122 (20 mg drug per kg body weight) were administered to six rats for toxicological diagnostic reasons. After two, four, and six weeks, the rats were dissected and blood, adipose tissue, brain, heart, lung, spleen, liver, kidneys, muscle, and hairs were collected. Adipose tissue was homogenized in and extracted by acetonitrile. For preparation of the other tissues, they were homogenized in water and treated with acetonitrile. The hair samples were first washed twice with water and once with acetone, then pulverized, and extracted with ether/ethyl acetate after alkaline hydrolysis with aqueous sodium hydroxide. JWH-018-d_9 (0.25 ng abs.) was added before each extraction as internal standard. The samples were analyzed by LC-MS/MS, MRM/ESI^+ (Thermo Fisher TSQ Quantum Ultra AM). Separation was achieved using a C18 column (150 x 2.1 mm, 3.5 µm) and gradient elution (0.1% aqueous formic acid and 0.1 % formic acid in acetonitrile). The analytical method for adipose tissue was fully validated in analogy to the guidelines of the Society of Toxicological and Forensic Chemistry. For the other tissues and hairs a partial validation for qualitative analysis with respect to selectivity, limit of detection, matrix effects, recoveries, and process efficiencies was performed.

Results: Two weeks after single drug administration, JWH-210 was still present in adipose tissue (96 ng/g), heart (0.59 ng/g), lung (0.66 ng/g), spleen (0.49 ng/g), kidneys (0.46 ng/g), hairs (0.15 pg/mg), and traces in blood. After four weeks, JWH-210 was found in adipose tissue (114 ng/g), spleen (1.3 ng/g), kidneys (3.0 ng/g), and traces in muscle and hairs. Six weeks after the administration, the synthetic cannabinoid could only be detected in adipose tissue (1.1 ng/g). JWH-122 was only present in adipose tissue after two (0.3 ng/g), four (23 ng/g), and six weeks (traces).

Conclusion: The present work proves that the two synthetic cannabinoids JWH-210 and JWH-122 incorporate in adipose tissue and are still present there after six weeks. The discrepant amounts of the two drugs indicate a different incorporation in adipose tissue. JWH-210 also persists in spleen and kidneys up to four weeks.

Keywords: Synthetic Cannabinoids, Rat Tissues, LC/MS/MS

S29

An Evaluation of Beta-Hydroxybutyrate (BHB) in Post-Mortem Blood as a Diagnostic Biomarker for Alcoholic Ketoacidosis

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Introduction: In chronic alcoholics sudden death may occur where the cause of death cannot be determined. Findings at post-mortem may be limited to only a fatty liver with a low or absent blood alcohol. Beta-hydroxybutyrate (BHB), along with acetone and acetoacetate, are ketone bodies produced through the breakdown of fatty acids during periods of fasting and malnutrition. A build-up of ketone bodies can lead to ketoacidosis, which is characterised by an acidic blood pH. In many cases of alcoholism, malnutrition is present and this, together with the oxidation of ethanol and extracellular fluid volume depletion arising from vomiting and decreased fluid intake, can lead to a type of ketoacidosis known as alcoholic ketoacidosis (AKA).BHB is considered a diagnostic biomarker for AKA along with the presence of acetone as an initial indicator of AKA.

Objective: To evaluate BHB as a diagnostic biomarker of alcoholic ketoacidosis.

Method: Post-mortem blood samples (N=474) submitted for routine toxicological analysis were analysed for the presence of BHB using an in-house validated method utilising gas chromatography-mass spectrometry (GC-MS).The cases selected included those where ketoacidosis was suspected or confirmed as contributing to the cause of death and a large cohort of control cases. Post-mortem records were reviewed and the cases grouped according to the cause of death and whether the deceased had a history of alcohol abuse or diabetes. The BHB results were compared with those for acetone and ethanol and categorised by case type in order to investigate the significance of BHB as a diagnostic biomarker for AKA.

Results: 41% of the cases had a previous history of alcohol abuse, 6% were previously diagnosed with diabetes and 2% had a history of both alcohol abuse and diabetes. The remaining 51% of cases had no history of alcohol abuse. Thirteen cases had BHB concentrations >250 mg/L (and acetone concentrations >250 mg/L) and the cause of death in all cases was attributed to alcohol abuse and fatty degeneration of the liver, however only one case specifically states AKA.

Seven cases with no previous history of diabetes or alcohol abuse had BHB concentrations in excess of 150 mg/L. The cause of death attributed to these cases, prior to the analysis of BHB was suicide (N=1), natural (N=5) and drug abuse (N=1). A BHB concentration of 728 mg/L was measured in the death attributed to drug abuse.

Conclusion: This study highlights the importance of testing BHB in all cases where the individual has a history of alcohol abuse or diabetes and where the cause of death is unascertained following the post-mortem examination. Although 20 cases in this study had BHB levels >250mg/L, only 2 cases were attributed to AKA suggesting this condition may be underreported.

Keywords: Beta-Hydroxybutyrate, Alcoholic Ketoacidosis, Alcohol

S30
Betahydroxybutyric Acid in Postmortem Fluids as an Indicator of Ketoacidosis

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Introduction: Ketoacidosis is a serious medical condition that may result from uncontrolled diabetes, chronic alcoholism, poor nutrition and ketogenic diets. There are assays available to quantify ketone bodies in serum and urine from living patients, but these techniques are not compatible with postmortem blood and vitreous fluid. A recent publication from Elliott et al. indicated that approximately 30% of undetermined deaths had elevated concentrations of ketone bodies in postmortem blood or vitreous fluid. Among these, the most stable and specific indicator of ketoacidosis was betahydroxybutyric acid (BHB).

Objective: A method was developed to quantify BHB in blood and vitreous fluid in support of postmortem investigations.

Method: Deuterated BHB was added as internal standard. Blood and vitreous specimens were prepared by protein precipitation and derivatized with BSTFA. Analysis was performed by Gas Chromatograph/Mass Spectrometer with a DB-17 column. Selected ion monitoring was used for quantitation. The method produced between-run variability less than 5% and accuracy within 11%. BHB was found to be stable in blood from gray top tubes for 2 days at room temperature and 14 days refrigerated.Candidate cases were identified by polling postmortem blood samples for positive acetone results. Submitting agencies were contacted to determine whether BHB analysis was beneficial.

Results: During April, 2013, there were 64 postmortem cases positive for acetone. Approximately 20% (n=13) were submitted for BHB analysis. The remaining cases were characterized as cause of death already known (33%, n=21), BHB not needed (23%, n=15), no reason given (9%, n=6) and no response received (14%, n=9). Results from the 13 cases with BHB analysis are included in the table below.

Age	Sex	BHB (mcg/mL)	Acetone (mg/dL)	Isopropanol (mg/dL)	Glucose in Vitreous (mg/dL)	History
12	M	Failed	22	5.7	490	new onset diabetes
44	M	1100	38		500	Unknown
56	M	1000	70			Unknown
65	M	890	25			Apparent natural
52	M	710	27			Unknown
56	M	590	32	5.1	96	Unknown
90	M	470	15			Apparent natural
	M	440	5.6			found @ truckstop
65	F	270	6.7		262	Unknown
50	F	240	17	10		found in bed
63	M	140	6.9			Suicide, struck by train
42	M	110	6.3			Unknown
27	F	0	5	9		Perforated rectum

Conclusion: As expected, there is a weak correlation between BHB and acetone (R^2=0.66). However, it is necessary to quantify BHB because acetone may be present without ketoacidosis. The gold standard for identifying diabetic ketoacidosis has historically been a vitreous glucose concentration above 200 mg/dL. Three cases in the table above had vitreous glucose results consistent with hyperglycemia, which complemented the elevated BHB results. There was one case with a normal glucose concentration in the presence of elevated BHB. Other findings in this case suggested degradation of the vitreous fluid (i.e., potassium greater than 20 mmol/L). This case demonstrates the risk of false negative results for ketoacidosis based on vitreous glucose alone. While vitreous glucose adds strong interpretive value when it remains elevated, BHB provides a more reliable and stable indicator of ketoacidosis.

Keywords: Postmortem, Ketone, Diabetes

S31
Clinical and Pathological Findings in Fatal Cases Involving the Ingestion of Methylone

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Introduction: The Miami-Dade County Medical Examiner Department has investigated eighteen deaths in which methylone was identified by the Toxicology Laboratory during a routine drug screen. Methylone, a cathinone derivative and beta-keto analog of methylenedioxymethamphetamine, MDMA, is an enactogen and stimulant often sold as components in "bath salts" or as a replacement for MDMA in "Molly" capsules. Its prevalence in Medical Examiner cases in Miami, Florida has steadily increased from two cases for the entire year of 2011 to nine cases within the first five months of 2013. Of the death investigation cases received over the last three years, four were classified as suicides, seven were classified as homicides, and seven were classified as accidents. Four of the accidental deaths were directly related to the ingestion of methylone and its toxic effects.

Objective: The objective of the presentation is to provide a comprehensive overview of the four cases in which methylone contributed to the cause of death. The review will describe each of the decedent's behavior leading up the terminal event, their clinical presentation to the hospital, and the pathological findings at autopsy. The information will provide valuable insight regarding the varying effects of methylone intoxication to forensic toxicologists, pathologists, medico-legal death investigators, and law enforcement personnel.

Method: Routine toxicological analyses were performed on post-mortem specimens, or ante-mortem specimens when available. All cases received a volatile analysis by headspace GC-FID. Urine specimens were analyzed by EMIT and also subjected to a liquid-liquid extraction for GC-MS analysis. Blood specimens were analyzed by ELISA and also subjected to a solid-phase extraction for analysis by GC-FID, GC-TSD, and GC-MS. Quantitation was performed by an outside reference lab. Case files were reviewed for each decedent in which methylone was identified and included in the cause of death. The review included case demographics, investigative information, social history, hospital reports, and pathological findings at autopsy.

Results: In all four cases, the decedents were at social events allegedly using MDMA or "Mollies". All decedents prior to death were incoherent, agitated, combative, exhibited altered mental status, and hallucinating. At hospital admission, all four decedents presented with diaphoresis, dilated pupils, elevated body temperature, tachycardia with hypotension, and severe metabolic acidosis. In one case, the decedent experienced seizures and cardiac arrest during transport. He suffered severe anoxic encephalopathy and subsequently died after prolonged treatment and hospitalization. Two of the four cases presented to the hospital in cardiac and respiratory arrest. At autopsy, one decedent exhibited petechial hemorrhages of the cortex and right cerebral hemisphere, while the other decedent did not present with any significant anatomical findings. The final decedent developed shock, rhabdomyolysis, and disseminated intravascular coagulation (DIC) in the emergency room and subsequently died.

Conclusion: In all four decedents, methylone was identified in the ante-mortem blood and/or urine specimens. Other drugs not administered by the hospital that were identified include diphenhydramine, doxylamine, ibuprofen, diazepam, butylone, and amphetamine. Methylone was listed as the cause of death or was listed as contributing to the cause of death for each of the cases described.

Keywords: Methylone, Bath Salts, Mollies, Disseminating Intravascular Coagulation (DIC)

S32
The Impact of Oxycodone Abuse on Miami and the State of Florida: 2000-2012

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Introduction: Over a ten year period (year 2000-2010), the Toxicology Laboratory at the Miami-Dade Medical Examiner Department (MDME) observed a substantial increase in the number of decedents in which oxycodone was identified. This increase correlated with a prescription drug epidemic that emerged across the entire nation during that last decade. The state of Florida was deemed the epicenter of this nationwide epidemic largely due to the prevalence of pain management clinics, or "pill mills" that sold medically unnecessary pain-killers for cash, often to out-of-state buyers who took the pills back to their home state for resale. The political and public health issues that resulted from this explosion of oxycodone abuse throughout the south generated new state legislation that ultimately impacted law enforcement, death investigation, hospitals and the public's perception of prescription drug abuse. Has this epidemic subsided or are we at a plateau that will continue for many years? Are new drugs replacing the old or are the old drugs becoming new again?

Objective: The objective of this presentation is to discuss the impact of this epidemic on the MDME and other medical examiner districts throughout the state. Statistics on the number of deaths across the state will be presented in addition to a discussion of how Florida dealt with the problem over time. Also discussed will be recent changes as an outcome of new legislation and changing drug abuse in Miami.

Method: Results for this study were obtained from the files of the MDME and the Florida Department of Law Enforcement's annual statistical report on *Drugs in Deceased Persons*.

Results: The incidence of oxycodone involved deaths at the MDME Department remained below 30 per year throughout the 1990's but had shown a steady yearly increase through 2009. The number of deaths peaked in 2010 with 108 cases. Due to the rampant abuse of oxycodone in the years leading up to 2010, Florida enacted new legislation to regulate pain management clinics. Legislation was intended to tighten rules for prescription writing, to reduce the abuse of oxycodone by penalizing doctors who over-prescribe, and to require prescribing doctors to utilize a centralized computer system. Following 2010 the incidence of oxycodone in deaths investigated at the MDME dropped by 40% suggesting the new legislation has had a positive impact on this epidemic. Since 2010 other drug abuse trends have begun to emerge however, including the return of heroin abuse.

Conclusion: The study demonstrates a rise in oxycodone detected in decedents by the MDME toxicology laboratory from 2000 through 2010 followed by a decrease from 2010 through 2012. This same pattern has been observed by medical examiner districts representing major metropolitan areas throughout Florida.

Keywords: Oxycodone, Heroin, Florida, FDLE

S33

Visualisation of Cocaine Metabolite (Benzoylecgonine) Depots in Oral Tissue Using an Immunohistochemical Staining Procedure

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Introduction: Oral fluid (OF) is gaining wide popularity as a non-invasive medium for monitoring drug use. Initially, the presence of drugs in OF was based on a concept that drugs circulating in the blood passed into oral fluid in relation to their concentration in the circulating blood. It has been noted however that in some drug users, the concentrations of drugs in OF greatly exceeds the concentrations measured in blood leading to a proposition that drugs may accumulate and form depots in oral tissue. The phenomenon of elevated drug concentrations in oral fluid has been well documented however little is known about the physiology of drugs in the oral cavity to help explain the observations. The measurement of high drug concentrations in OF and our limited understanding of drug elimination via OF poses a problem to the interpretation of analytical results.

Objective: The objective of this study was to explore how high drug concentrations form in the mouth and OF. The study focuses on using immunohistochemical techniques to enable the detection of drugs bound within cells and tissues to be visualised by antibody-antigen reactions.

Method: Porcine tongues were exposed to solutions of 50 ng/mL, 100 ng/mL, 250ng/mL, 500 ng/mL, 1 µg/mL and 10 µg/mL of cocaine and smoke from 'crack' cocaine. After exposure to the cocaine solutions, the tongues were thoroughly washed using several water washes. Transverse sections of tongues together with non-exposed tongues (controls) were fixed in 10 % neutral-buffered formalin (NBF) for 48 hours. Tissue was then embedded in paraffin prior to sectioning.

Following 24 hours incubation, sections were de-paraffinised and re-hydrated through graded alcohols. To avoid non-specific binding (NSB) of the antibody an avidin-biotin block was applied. Dulbecco's modified Eagles medium block was used to inhibit NSB caused by epitopes present in tissue samples prior to applying a primary monoclonal benzoylecgonine antibody at 0.35 µg/mL for 24 hours. After incubation, a rabbit-anti-mouse antibody was applied for two hours to act as the biotinylated secondary antibody before applying a avidin-biotin-peroxidase complexes. Tissue sections were stained using a 3, 3' diaminobenzidinetrahydrochloride (DAB) before counterstaining sections with Mayer's haemotoxylin. Finally tissue slides were dehydrated using graded alcohols, cleared and mounted in *Pertex.*

Results: Positive staining using a benzoylecgonine antibody is characterised by the formation of a brown colour caused by the reaction between DAB and the peroxidases from the avidin-biotin-complexes. Positive staining could be detected at all cocaine exposure concentrations as well as in tongues exposed to 'crack' cocaine smoke. Staining is clearly visualised in the epithelium and the lamina propria as well as further into the muscular tissue of the tongue. Future work will explore whether the degree of staining is dose related. *Results will be shown presented pictorially.*

Conclusion: Drugs that are smoked or taken by nasal insufflation have the potential to enter mouth tissue by mechanisms other than via the blood. This study demonstrates the entry of cocaine in tongue tissue and provides support for the proposition that drugs can form depots in oral tissue from which they may be subsequently released over time.

Keywords: Oral Fluid, Tissue Depots, Cocaine, Immunohistochemical Staining

S34

Issues in Interpreting Hair Findings in Children: Bias by Contamination?

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Introduction: The differentiation between drug use and external contamination has been frequently referred to as one of the limitations of drug testing in hair.

Objective: The detection of relevant metabolite(s) has been proposed to minimize the possibility of external contamination causing a misinterpretation. Difficulty arises when a metabolite is not detected either due to the absence of specific metabolite or to low doses of the drug being used. We were recently involved in such a case, involving a young child potentially exposed to carbamazepine (child custody case).

Method: In various papers, the concentrations of carbamazepine in the hair of adult patients under daily therapy are > 10 ng/mg. However, nothing has been published about the detection of carbamazepine in the hair of children and there is no controlled study to interpret the analytical findings.

Results: The following carbamazepine concentrations were measured by LC-MS/MS in the hair of a 21-month old girl: 154 (0-1 cm), 198 (1-2 cm), 247 (2-3 cm) and 368 pg/mg (3-4 cm) after decontamination.

Obviously, the concentrations measured in the hair are much lower than those observed in patients under daily treatment. In that sense, the frequency of exposures appears as infrequent (low level of exposure), with marked decrease in the more recent period. However, the girl was never prescribed carbamazepine and the mother, who was under carbamazepine therapy, denied any administration.

As a consequence, contamination was considered as an issue and interpretation of the results was a challenge that deserves particular attention.

There are many differences between the physiology of hair from children and those from adults: hair from children is thinner and more porous, the ratio of anagen and catagen phases is not maintained, and the growth rate can be different, at some periods, from the usual 1 cm/month.

At least, three possible interpretations of the measured carbamazepine concentrations could have been addressed:

1. decrease in administration in the more recent period;
2. increase of body weight of the child due to growing, so the same dosage will result in lower concentrations in hair; and
3. sweat contamination from the mother at the time the girl is with her in bed, the older hair being longer in contact with the bedding.

Conclusion: In this case, it was impossible to conclude that the child was deliberately administered carbamazepine. The results of the analysis of hair could indicate that she was in an environment where carbamazepine was being used and where the drug was not being handled and stored with appropriate care.

In view of these results we concluded that a single determination should not be used firmly to discriminate long-term exposure to a drug.

Keywords: Hair, Carbamazepine, Child, Interpretation, External Contamination

S35

The Effects of Hemp Oil Consumption on Hair Drug Testing for the Presence of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THCA)

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Introduction: Hemp Oil is used for its nutritional value specifically as a source of omega fatty acids. It can be purchased at many health food stores as well as via the internet. Consumer awareness of delta-9-tetrahydrocannabinol (THC) levels in Hemp Oil is still in question. Previous studies have quantitated the presence of THCA metabolite in urine specimens. THCA levels in hair specimens after hemp oil consumption has not been evaluated.

Objective: The proposed US Substance Abuse and Mental Health Services Administration (SAMHSA) guidelines for Federal Workplace Drug Testing in hair recommend a cutoff level of 0.05 pg/mg for THCA. The objective of this study was to administer hemp oil products to THC free volunteers and determine the THCA levels in head hair samples. The concentrations determined will be used to evaluate the recommended SAMHSA cutoff.

Method: Eleven volunteers, 6 males and 5 females, were given daily doses of hemp oil products over a 30 day period. Hair color, cosmetic treatments and ethnicity were documented. All subjects' hair specimens determined to be negative for THCA prior to the start of the study. Four different Hemp Oils were used: Manitoba Harvest Hemp Oil Softgels (1000 mg), Manitoba Harvest Hemp Oil, Nutiva Organic Hemp Oil, and Foods Alive Organic Hemp Oil. Dosing was approximately 15 ml/day as recommended by the manufacturer. After 45 days the subjects' hairs were recollected and the first half inch segment of hair closest to the scalp was analyzed for THCA. After washing with methanol, analysis was performed by solid phase extraction followed by 2-dimensional GC and negative chemical ionization MS/MS. Additionally, the hemp oil products were evaluated for THC content using liquid/liquid extraction and LC/MS/MS analysis.

Results: Of the eleven volunteers, 8 hair specimens where negative for THCA at a LOQ of 0.02 pg/mg. Three individuals were determined to be positive for THCA at 0.03, 0.04, and 0.05 pg/mg of hair. The hemp oil products containedthe following THC concentrations: Manitoba Harvest Hemp Oil Softgels (1.7 ug/mg), Manitoba Harvest Hemp Oil (0.9 ug/mg), Nutiva Organic Hemp Oil (4.1 ug/mg), and Foods Alive Organic Hemp Oil (3.1 ug/mg). The positive hair test results did not correlate in any manner to the concentrations of THC in the hemp oil products or hair color and cosmetic treatments. Differences due to ethnicity could not be evaluated with the study population.

Conclusion: The daily use of Hemp Oils may result in the detection of low levels of THCA in hair samples and may cause positive drug test results at the proposed Federal Workplace Drug Testing cutoff level of 0.05 pg/mg. Further testing on a wider cross section of commercially available hemp oil products and the effects of dosing regiments needs to be explored.

Keywords: Hemp Oil, Positive Drug Test Cutoff Level, THCA

S36

Nonlinear Plasma Pharmacokinetics of MDMA and Major Metabolites in Rats and Accompanying Short-Term Pharmacodynamic Effects

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Introduction: Extrapolation of (±)3,4-methylenedioxymethamphetamine (MDMA) findings from animals to humans requires assessment of pharmacodynamics and pharmacokinetics at multiple doses in various species. A wealth of pharmacodynamic data showed that MDMA's neurochemical, endocrine and behavioral effects occur at similar doses (i.e., 1-2 mg/kg) in rats and humans. However, there are few and inconsistent data comparing pharmacokinetics at comparable doses in these two species.

Objective: To evaluate MDMA pharmacokinetics in rats receiving 2.5, 5 and 10 mg/kg MDMA by the subcutaneous (sc) route, and to monitor the serotonin behavioral syndrome and core temperature.

Method: Blood was collected by peripheral venous catheter for 24 h (pre-dose and 0.5, 1, 2, 4, 6, 8, 16 and 24 h post-dose). Plasma specimens (0.1 mL) were analyzed for MDMA and metabolites (±)-3,4 dihydroxymethamphetamine (HHMA), (±)-4-hydroxy-3-methoxymethamphetamine (HMMA), (±)-3,4-methylenedioxyamphetamine (MDA) and (±)-4-hydroxy-3-methoxyamphetamine (HMA) by liquid chromatography tandem mass spectrometry (LCMSMS). Linearity was from 5-10 to 1,500 µg/L. Serotonin behavioral syndrome (flat body posture, forepaw treading, ambulation and weaving) and core body temperature were monitored at blood collection time points.

Results: After 2.5 mg/kg sc, mean±SD maximum MDMA concentration (C_{max}) was 164±47.1 ng/mL, similar to human peak levels following oral MDMA. At this MDMA dose, HHMA and HMMA were the predominant metabolites, with only 20% metabolized to MDA. After 5 and 10 mg/kg, MDMA areas under the curve (AUC) (879±133 and 2,880±492 h*ng/mL) were 3- and 10-fold greater than after 2.5 mg/kg (272±71.6 h*ng/mL), respectively. MDA also had 5.5- and 24.4-fold AUC increases after the same doses (47.4±23.5 h*ng/mL at 2.5 mg/kg, 262±83.3 at 5 mg/kg and 1,158±244 at 10 mg/kg). In contrast, HHMA and HMMA AUC values after 5 and 10 mg/kg (790±194 and 970±247 h*ng/mL for HHMA, and 1,442±430 and 2,133±654 for HMMA) were 1.4 and 2-fold greater than those following 2.5 mg/kg 532±97 h*ng/mL for HHMA and 1,032±161 for HMMA. Therefore, non-linear pharmacokinetics were documented for MDMA at high doses (5 and 10 mg/kg; $P<0.05$). Core temperature was statistically significantly higher at 10mg/kg MDMA compared to vehicle, 2.5 and 5 mg/kg doses. Ambulation, flat body, treading and weaving scores were significantly higher after 10mg/kg; weaving scores also were significantly higher after 5 mg/kg MDMA. Total serotonin behavioral syndrome acute effects peaked at time of C_{max} (T_{max}) for MDMA, and core temperature peaked at MDA $T_{max.}$

Conclusion: Given the important similarities between MDMA pharmacokinetics and pharmacodynamics in rats and humans, data from rats may be clinically relevant when similar doses and appropriate routes of administration (sc in rats and po in humans) are employed.

Keywords:MDMA, Comparing Pharmacokinetics Doses, Similarities in Rats and Humans

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S37

Is CYP2D6 Pharmacogenetics Involved in P-Methoxymethamphetamine (PMMA) Toxicity?

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Introduction:A severe outbreak of 29 fatal poisonings related to the designer drug para-methoxymethamphetamine (PMMA) occurred in Norway in 2010-2013. The fact that some individuals suffer fatal intoxication while others with similar drug exposure survive, suggests that there may be individual differences in the vulnerability to the toxic effects of PMMA. One explanation for this observed variation in susceptibility could be genetic variability in the PMMA metabolism. PMMA is mainly metabolized by the polymorphic cytochrome P450 (CYP) 2D6 enzyme to the active metabolite p-hydroxymethamphetamine (p-OHMA).

Objective: The objective of our study was to examine the individual CYP2D6 genotype profiles in fatal and nonfatal PMMA poisonings, to search for possible pharmacogenetic determinants for PMMA toxicity.

Method: The frequency of CYP variant alleles in

A. PMMA-related fatalities (N=25 available for analysis) were compared with three reference groups including
B. nonfatal PMMA-poisonings with PMMA blood concentrations ≥0.09 mg/L (N=30, forensic samples, mainly from subjects suspected of driving under the influence of drugs),
C. natural deaths (N=205) and
D. healthy blood donors (N=100), respectively.

In blood samples from group A, B and D, sequence variants of CYP2D6 (*1 (wt), *3, *4, *6) were analyzed by real-time PCR and melt curve analysis, and CYP2D6 gene copy numbers were determined by pyrosequencing and quantitative real-time PCR. For Group C, all analyses were performed by pyrosequencing (previously published data by Zackrisson AL 2004).

Results: The variant allele frequency of CYP2D6 ultrarapid metabolizer (UM) genotypes was 8% in PMMA-fatalities, compared to 1%, 0.5% and 0% in blood donors, natural deaths and nonfatal PMMA-poisonings, respectively (p=0.20, Fishers test).

The frequency of CYP2D6 slow metabolizer (SM) genotypes in nonfatal PMMA-poisonings was significantly higher than in healthy blood donors and natural deaths (30.0% vs 3.0% and 7.8%, respectively, p=0.00, Chi square). The frequency of the SM genotype in *fatal* PMMA-poisonings (12.0%) was not significantly different from neither blood donors (p=0.09, Fishers test) nor nonfatal poisonings (p=0.11, Chi square).

The frequency of extensive metabolizers (EM) in nonfatal PMMA-poisonings was 36.7%, compared to 56% in blood donors (p=0.06, Chi square), 53.2% in natural deaths and 40.0% in PMMA-fatalities. For intermediate metabolizer (IM) genotypes, the frequencies were not significantly different between any of the groups (36.7%, 40.0%, 38.5% and 40.0%, respectively).

Conclusion: Our study suggests that the risk of PMMA toxicity may, at least partly, depend on CYP2D6 genotype. The SM genotype was significantly overrepresented in nonfatal cases, while a trend towards overrepresentation of the UM genotype in PMMA-fatalities and underrepresentation of the EM genotype in nonfatal cases was seen. This indicates that defective CYP2D6 genotypes, corresponding to the *SM* phenotype, might be protective while efficient CYP2D6 genotypes, corresponding to EM or UM phenotypes, might be associated with increased risk of toxicity. However, the power of our study is too low to detect a possible difference with significance.

Blood concentrations of PMMA and its metabolites in these fatal and non-fatal PMMA poisonings will also be presented at the SOFT meeting.

Keywords:PMMA, 4-Methoxymethamphetamine, Para-Methoxymethamphetamine, Cytochrome P-450 CYP2D6, Designer Drugs, Fatal Intoxication, Forensic Toxicology

S38
High-Throughput Analysis of Clenbuterol in Plasma Using LDTD-MS/MS

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Introduction: Clenbuterol is a β2-adrenergic stimulant used by sufferers of breathing disorders as a decongestant and bronchodilator. It is also a stimulant and thermogenic drug increasing aerobic capacity, central nervous system stimulation, blood pressure, and oxygen transportation. The quantitative determination of clenbuterol in the plasma matrix has particular interest for various fields such as clinical, forensic analysis, and doping controls.

Objective: To increase the throughput analysis of clenbuterol in different matrix, the LDTD-MS/MS technology was used. This technology uses the laser diode thermal desorption (LDTD) to transfer and ionize drug compound in a mass spectra. Typical run time takes 9 seconds per sample.

Method: A calibration curve and quality control material are spiked in the plasma matrix at 10 to 5000 pg/ml range. A solid phase extraction procedure is performed to isolate Clenbuterol before adding extracted sample in a specially constructed 96-well plate. The extracts were performed on SiliaPrep CleanDrug (1cc/100mg). After activation step, a mix of 500 µL of sample, 50 µL internal standards and 500 µL of sodium acetate buffer (100mM, pH6) were loaded. Following a serial wash with water, acetic acid solution (1N) and methanol, drugs were eluted with a mixture of ethyl acetate/isopropanol/ammonium hydroxide (80/20/2). Elution fractions were evaporated to dryness and reconstituted with 50 µL of methanol/water (75/25) then 2 µL is transferred to a 96-well plate (LazWell) for analysis. Samples were desorbed from the LazWell plate using the following Laser pattern: 3 seconds ramps to 45% laser power and 0% laser power in 0.1 sec. The carrier gas flow rate was 3 L/min of medical grade air. Molecules were ionized in positive mode with 3µA on corona needle. The MS/MS transition for Clenbuterol was 277 ->132 with a CE value of 25.

Results: The calibration curves show excellent linearity with r^2 0.9973 for the intra-assay run and r^2 0.9947, 0.9972 and 0.9975 for the inter-assay run. The accuracy and precision were evaluated at Low, Medium and High QC level (100, 500 and 2500 pg/ml). The accuracy and precision (6 and 18 replicates for intra and inter-assay, respectively, were used) between 93.4 to 101.7% and 1.4 to 9.5%, respectively were obtained. Following the extraction process, all samples were stored at 4°C to evaluate the wet stability of the drug. A wet stability of 72h was found with an accuracy and precision of 96.5% and 11.7% at the low standard concentration (50 pg/ml). The stability of dry samples at room temperature in LazWell plate was also determined. Dry stability of 48h was obtained with an accuracy and precision of 99.8% and 8.8% at the low standard concentration (50 pg/ml). Matrix effect was evaluated at 100 and 1000 ng/ml and an accuracy and precision (9 replicates) between 85.0 to 99.5% and 4.3 to 13.3%, respectively for six different matrices. Blank interference level of each matrix was evaluated and interference lower than 20% for low standard concentration (50 pg/ml) was reached.

Conclusion: LDTD technology provides an ultra-rapid analysis method for clenbuterol in plasma matrix. This extraction technique combined to LDTD-MS/MS analysis method demonstrates accurate, precise, specific, and stable results.

Keywords: Clenbuterol, LDTD (Laser Diode Thermal Desorption), Plasma Matrix

S39

The Evaluation of Laser Diode Thermal Desorption (LDTD) for High Throughput Analysis in Forensic Science

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Introduction: The laser diode thermal desorption (LDTD) source is a high throughput source that allows for ultra-fast sample introduction in mass spectrometry (MS) and can be interfaced to multiple MS platforms from a wide range of instrument manufacturers.As this is a supplemental instrument to expand the capability of an existing MS, it is less expensive for the laboratory to implement than an entire new MS platform.While this technique is finding successful application in many fields, its further use in forensic laboratories requires a careful consideration and demonstration of the validity of this technique.

Objective: The purpose of this study was to evaluate the applicability of LDTD in areas of forensic interest. Specifically, we evaluated the instrument's performance as a high throughput source coupled with MS for the detection of controlled substances, including newly emerging designer drugs, drugs used in facilitated drug crimes, and those relevant to postmortem toxicology. The LDTD does not use chromatographic separation therefore it is important to determine whether this technology provides sufficient sensitivity and selectivity to allow for screening and quantification of analytes. This project investigated the efficiency and cost effectiveness of this new technology, and the feasibility of it being implemented in forensic laboratories.

Method: Approximately forty drugs, selected from common drug classes such as antidepressants, amphetamines, and benzodiazepines were extracted from urine and blood by solid phase extraction (SPE) or liquid-liquid extraction (LLE). The samples were analyzed by a Phytronix LDTD source coupled to an AB SCIEX API-4000 MS operated in multiple reaction monitoring mode (MRM). The number of ion transitions monitored for each drug and internal standard were two and one, respectively. Linearity was established by analyzing six non-zero concentrations over five separate runs (n=5). Accuracy and precision were determined by analyzing QC samples at three concentrations (n=15). Other validation studies were performed to determine LOD, LOQ, interference, carryover and matrix effect.
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Results: The majority of drugs analyzed in this study produced LPA results that were comparable to those accepted in forensic toxicology laboratories. For example, validation results of benzoylecgonine in urine gave a between-run (n=15) and within-run (n=3) %CV of less than 16% and 20% respectively, and overall accuracy (n=45) of 95%. Results of diazepam in urine gave a between-run (n=15) and within-run (n=3) %CV of less than 16% and 19%, respectively and overall accuracy (n=45) of 104%. The use of MRM allows for selectivity of most analytes; however for some drugs further method development (e.g. derivitization) is needed in order to differentiate them from possible interferences.

Conclusion: The LDTD source is a promising new technology to provide forensic laboratories with an option for high throughput sample screening and quantification in the analysis of controlled substances and forensic toxicology. For the majority of drugs analyzed in this study, LDTD produces linearity ranges that are comparable to those obtained by LC-MS/MS. The lack of chromatographic separation can potentially cause problems with selectivity so careful method development and validation are critical to successful implementation of this technology.

Keywords: LDTD, High Throughput, Controlled Substances

S40
DART-MS for Rapid, Preliminary Screening of Urine for DMAA

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Introduction:1,3-Dimethylamylamine, known as DMAA, is a stimulant that has gained recent attention due to its use as a doping agent and an ingredient in workout supplements. Its increased prevalence on the market has resulted in a documented rise in emergency visits and calls to poison control centers, and has also been associated with instances involving cerebral hemorrhages and fatalities of users. Due to emerging information about health risks of this drug, DMAA has been under scrutiny by several governments, including Australia, Canada, the U.S., and New Zealand, where it has been identified as an abused substance.

Objective: Here, we report an ambient ionization mass spectrometry method, direct analysis in real time-mass spectrometry (DART-TOF-MS), used for the detection of DMAA in multiple nutritional supplements as well as directly in unprocessed urine.

Method: Solid nutritional supplements were tested directly as a solid powder or pill, without any preparation whatsoever. In addition, a volunteer ingested the supplement Ripped Juice EX2, containing an unknown amount of DMAA, with urine collected and tested for the presence of the drug. Urine samples were tested directly by DART-MS without any preparations and positively identified the drug over 36 h. Additionally, the raw urine samples were processed in two ways, followed by DART-MS analysis. First, the DMAA was liquid-liquid extracted from the urine with methylene chloride, testing the extract by DART-MS. Second, the urine was processed using DPX tips prior to DART-MS analysis.

Results: DMAA was successfully detected from urine in all sets of samples over 36 h, with liquid-liquid extraction giving the most robust response, followed by DPX tips, and the neat samples, respectively. However, testing the neat samples directly by DART-MS provided an instantaneous result, saving time and resources associated with sample processing as well as the time-savings associated with chromatographic separations.

Conclusion: The three processing methods provide an interesting decision point, providing instantaneous analysis with no sample preparations versus more robust responses related to increased levels of sample preparations. Ultimately, DART-MS proved useful in a rapid screening of DMAA in urine samples and demonstrates the technique as a viable preliminary method of analysis of drugs in biological matrices.

Keywords: DART-TOF-MS, Detection of DMAA, Rapid Screening of DMAA

S41

Chiral Separation of Plasma Amphetamines Enantiomers by LC-MS/MS with Precolumn Derivatization

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Introduction: Amphetamines are widely abused psychoactive substances, yet consumption of certain over-the-counter and prescription medications may yield positive amphetamines results. Chiral separation of l- and d-amphetamine and methamphetamine can help determine whether the source was licit or illicit.

Objective: To develop and validate an LC-MS/MS method to simultaneously determine l- and d-amphetamine and methamphetamine in plasma.

Method: 0.5 mL plasma was spiked with racemic d_{11}-amphetamine and methamphetamine internal standards, followed by 2 mL 1% formic acid in water. Samples were centrifuged and then loaded onto pre-conditioned Phenomenex® Strata™-XC Polymeric Strong Cation solid phase extraction (SPE) columns. After washing with 0.1M acetic acid and methanol, analytes were eluted with 5% ammonium hydroxide in methanol. The eluate was evaporated to dryness and reconstituted in 100 µL water. The derivatization was performed with 1-fluoro-2,4-dinitrophenyl-5-*l*-alanineamide (Marfey's reagent) and heating at 45°C for one hour. Derivatized enantiomer separations were performed under isocratic conditions (methanol:water, 60:40) with a Phenomenex® Kinetex® 2.6 µm C_{18} column. Analytes were identified and quantified by two MRM transitions and their ratio on a 3200 QTrap (AB Sciex) mass spectrometer in ESI- mode. Authentic plasma specimens were collected from an IRB-approved study that included controlled Vicks® VapoInhaler administration.

Results: All amphetamine analytes and internal standards were baseline resolved in <10 min followed by column washing and equilibration, yielding a 20 min run-time. Analyses were linear from 1-500 µg/L. Inter- and intra-day imprecision (%CV) and accuracy for 3, 30, and 300 µg/L control samples were 0.7-6.8% and 87-100%. Matrix effects and extraction efficiencies were -18.3-20.3% and 98.7-117.4%, respectively. Samples diluted 2- and 10-fold were 81-91% of target. No endogenous (n=10) or exogenous (n=18) interferences were identified. All analytes were stable for 24 h at 4°C on the autosampler. l-Methamphetamine was detected in authentic plasma specimens at 1.1-10 µg/L, following Vicks® VapoInhaler administration.

Conclusion: An LC-MS/MS method for the simultaneous detection of l- and d-amphetamine and methamphetamine in plasma was successfully developed and validated, and implemented to detect analytes after Vicks® VapoInhaler administration. The method was specific (no endogenous or exogenous interferences) and sensitive (LOQ 1 µg/L). Amphetamine and methamphetamine enantiomer separation was successfully achieved quickly (<10 min) by derivitization with Marfey's reagent and reverse-phase chromatography, eliminating the need for expensive chiral columns or chiral additives like cyclodextrins that are incompatible with MS detection.

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Keywords: Chiral Separation, d-Amphetamine, d-Methamphetamine, Plasma, LC-MS/MS

S42
Quantitation of Drugs of Abuse in Urine Using UHPLC-TOF Mass Spectrometry

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Introduction: Drugs of abuse are typically screened by immunoassay techniques (EMIT) and confirmed by GC-MS or LC-MS-MS assays. We present an alternative route for analysis, quantification and confirmation of these drugs using a Time-of-flight (TOF) mass spectrometer. The newer detector technology TOFs which allow for wider dynamic range can be used for quantitative analysis of target drugs similar to the quadrupole instruments. However, unlike quadrupole instruments that are not very sensitive in the scanning mode, TOF can be used for qualitative screening of unknown compounds. The part-per-million mass accuracy capability provided by the TOF along with retention time matching without the need for fragmentation information can be used to identify and confirm the presence of both known and unknown target analytes.

Objective: Quantitative and qualitative analysis of several classes of drugs of abuse in urine using UHPLC-TOF.

Method: A PerkinElmer Flexar™ FX-10 LC pump with a PerkinElmer AxION® 2 TOF was used for UHPLC separation and detection of the drugs. The separation was achieved on a PerkinElmer Brownlee SPP C-18, 2X50 mm, 2.7 µm column using a mobile phase gradient of water and acetonitrile containing 0.1% formic acid. The TOF was operated in positive mode (*m/z* 100 - 1000) at 3 spectra/sec in the sensitive TrapPulse mode. Urine was spiked with varying concentrations of the analyte drugs along with internal standards (d5-diazepam and d3-doxepin). Urine was extracted over solid phase cartridges prior to analysis. A PerkinElmer Ultraspray2™ dual ESI ion source was used with a commercial mass calibrant mix infused through the second probe.

Results: Separation of nearly 20 drugs of abuse belonging to several classes including opiates, benzodiazepines, amphetamines, and cannabinoids was achieved within 7 min. Greater than 80% recovery was achieved for majority of the drugs upon SPE extraction. Limit of detection for most drugs was between 1-5 ppb, well within the cut off required by the EMIT assay. All of the calibration curves were linear with $r^2 > 0.99$. Besides screening for target analytes, the data was mined for non-target compounds. A database software search tool helped identify caffeine and its metabolites based on accurate mass and isotope ratio information. The presence of these non-target compounds was confirmed by retention time matching with standards.

Conclusion: UHPLC-TOF analysis results in LODs of 1-5 ppb for majority of the drugs of abuse analyzed in urine, well below regulatory limits. The ADC technology fitted in the AxION 2 TOF allows for wide linear dynamic range. Using the accurate mass capability of the AxION 2 TOF and the database search capabilities of AxION EC ID software, can identify unknown drugs of abuse in urine.

Keywords: Drugs of Abuse, Time-of-Flight Mass Spectrometry, Urine, UHPLC

S43
Stability of Alpha-PVP in Serum, Whole Blood and Urine

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Introduction: Pyrovalerone is a central nervous stimulant used to treat chronic fatigue and as an appetite suppressant. It is a schedule V drug in the US and is rarely prescribed due to its abuse potential. Recently drug users have begun illicitly using similar drugs that are not controlled. Alpha-PVP has gained popularity for this purpose over the past several years necessitating the development of analytical methods to detect this drug in biological fluids.

Objective: A liquid chromatography-tandem mass spectrometry test for the quantification of alpha-PVP in blood, serum and urine was developed and validated. During validation it became evident that alpha-PVP is unstable in serum, which was initially being used as the calibration and control matrix. Additional stability studies were performed to identify and quantify this instability and to investigate the stability in other matrices.

Method: Serum, blood and urine specimens were fortified with alpha-PVP, stored under various conditions and analyzed. Quantification was achieved by using hand-spiked calibration curves. Instability was measured by comparing daily concentrations to those determined on the first day of validation.

Results: During the initial validation, calibrators were prepared by spiking blank serum samples with alpha-PVP and using pooled serum, blood and urine controls that were stored at -70^{o}C for 9, 21 and 21 days, respectively, prior to analysis. The measured concentrations of these controls were 30-75% below nominal values. Based on previous experience additional controls were made using sodium citrate preservative. Acidifying samples did not sufficiently improve the stability of alpha-PVP in serum . It was noted during this portion of the study that the hand-spiked serum calibrators seemed to deteriorate between the time of spiking and the time of extraction. The concentration of alpha-PVP in low (6.0 ng/mL) and high (150 ng/mL) serum controls spiked 7 hours prior to the preparation of the calibration curve and extraction had a mean concentration of 1.9 and 45 ng/mL, respecitivly. In comparison, controls spiked concurrently with the calibration curve and immediately extracted had average concentrations of 6.1 ng/mL (low) and 156 ng/mL (high). A similar experiment was performed in blood and no loss of analyte was indicated. Finally a real patient blood specimen was evaluated. The sample was analyzed and a concentration of 501ng/mL was obtained. The sample was then stored at room temperature, refrigerated (3^{o}C) and frozen (-10^{o}C) and reanalyzed on days 1, 3, 7, 15 and 35. The results are summarized below:

Storage Condition	Day 1	Day 3	Day 7	Day 15	Day 35
Room Temp	534	544	441	475	237
Refrigerated (3^{o}C)	555	557	542	538	513
Frozen (-10^{o}C)	588	538	523	537	500

Conclusion: Alpha-PVP is unstable in serum, blood and urine under most storage conditions. Acidifying specimens can improve stability but does not completely address the problem. Blood is the preferred matrix for quantitative analysis and it is recommended that the specimens be acidified and stored in the refrigerator to improve the interpretive value of the result.

Keywords: New Psychoactive Substances, Alpha-PVP, Stabililty

S44

Identification of In Vivo and In Vitro Metabolites of UR-144 and XLR-11 by UPLC-QTOF Mass Spectrometry

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Introduction: Cannabimimetic compounds are sprayed onto plant material and sold for use as recreational drugs despite their adverse effects. Two compounds that have been identified in herbal products are the tetramethylcyclopropyl ketone indoles UR-144 and XLR-11 (fluorinated UR-144).

Objective: This study aimed to identify in vivo and in vitro metabolites of UR-144 and XLR-11 to provide the forensics community with suitable markers of use.

Method: Male ICR mice were dosed with 3 mg/kg of UR-144 and XLR-11 via intravenous injection. They were placed in metabolism cages and their urine collected over a 24 h. period. Urine from mice dosed with the same compound was pooled for analysis. In vitro samples were generated by incubating the compounds at 100 µM in cryopreserved human hepatocytes. At 0, 15 and 120 min an aliquot of 100 µL were removed and quenched with acetonitrile containing 0.2% acetic acid. Both in vivo and in vitro samples were hydrolyzed with 25 µL of 12.3 units/ µL of beta-glucuronidase in 0.1 M ammonium acetate and incubated for 3.5 h at 60°C. In vivo samples were then subjected to a salting-out assisted liquid-liquid extraction (SALLE).

Data were collected on both the hydrolyzed and unhydrolyzed samples using a Waters Acquity ultra performance liquid chromatography (UPLC) system coupled to a Synapt G2 HDMS quadrupole time-of-flight (QTOF) mass spectrometer. All data were acquired using a MS^E method, which acquires both low and high energy data simultaneously. Liquid chromatography was carried out using an Acquity BEH C18 column (1.7 µm X 2.1 X 50mm) connected to a Vanguard BEH C18 pre-column (1.7 µm X 2.1 X 5 mm) and held at 30 °C. A gradient elution with a flow rate of 500 µL/min was used with mobile phase A consisting of water with 0.1% formic acid and mobile phase B consisting of acetonitrile with 0.1% formic acid.

Results: Both UR-144 and XLR-11 were extensively metabolized with no parent compound remaining in the mice urine. Parent compounds were present in the in vitro samples. Monohydroxylation leading to glucuronidnation represented the majority of the metabolites detected. Monohydroxylation occurs on the tetramethylcyclopropyl group and on the indole portion of the molecule. Dihydroxylated metabolites were also seen consisting of monohydroxylation of both the tetramethylcyclopropyl group and the indole side of the molecule. In addition, XLR-11 underwent defluorination, resulting in several metabolites common to both parent compounds. Major metabolites were consistent between the in vivo and in vitro data.

Conclusion: This research provides forensic practitioners with the identification of major and minor in vivo and in vitro metabolites of novel designer drugs, UR-144 and XLR-11. Major metabolic transformations are the same for both compounds in the in vivo and in vitro samples. Due to defluorination of XLR-11, several metabolites are common to both compounds, and therefore should not be used for unambiguous identification.

Keywords: XLR-11, UR-144, Metabolites

S45

Drug Screening in Medical Examiner Casework by High Resolution Mass Spectrometry (UPLC-MS^E/TOF)

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Introduction: Postmortem drug findings yield important analytical evidence in medical examiner casework, and chromatography coupled with nominal-mass spectrometry (MS) serves as the predominant general unknown screening approach. High resolution mass spectrometry is now available and may further advance drug screening in the medical examiner setting.

Objective: To evaluate a postmortem drug screening method by ultra-performance-liquid-chromatography (UPLC) coupled to hybrid quadrapole time-of-flight mass spectrometer (MS^E/TOF), with comparison to validated nominal mass UPLC-MS and UPLC-MS/MS screening methods in routine use.

Method: The UPLC-MS^E/TOF method screens for over 950 toxicologically-relevant drugs and their metabolites including all major CNS drugs except for the cannabinoid and acidic agents. UPLC-MS^E/TOF analysis was performed in full-spectrum (m/z 50-1000) mode using an MS^E acquisition of both molecular and fragment ion data at low (6eV) and ramped (10-40 eV) collision energies. Blood was prepared by solid phase extraction and co-analyzed by UPLC-MS, UPLC-MS/MS and UPLC-MS^E/TOF screening methods. Validation for UPLC-MS^E/TOF mass error, limit of detection, reproducibility of detection, column recovery, matrix effect was performed with large matrix-matched panels of drug and metabolite reference material. A cohort of 300 medical examiner samples were co-analyzed by nominal and high resolution methods for evaluation of drug and metabolite detection in routine casework.

Results: Mass error averaged 1.27 PPM for a panel of 81 reference drugs and metabolites. Lower limit of detection by UPLC-MS^E/TOF ranged from 0.5-100 ng/mL and compared closely with UPLC-MS/MS analysis. Influence of column recovery and matrix effect on limit of detection was determined, with low detection limits for drugs and metabolites demonstrating column recovery greater than 80% and matrix effect less than 20%. Column retention time correlated with ion suppression and major matrix effect was observed with early eluting drugs such as nicotine, morphine, hydromorphone and also with late eluting agents such as clomipramine and amiodarone. Limited effect on recovery and matrix interference has been observed in decomposed case samples following our solid phase extraction method. Drug and metabolite detection by UPLC-MS^E/TOF was compared to UPLC-MS and UPLC-MS/MS findings for postmortem blood analysis in 300 medical examiner cases. Positive findings by all methods totaled 1528 with detection rates of 57% by UPLC-MS, 72% by UPLC-MS/MS and 80% by combined UPLC-MS technologies. Compared to nominal mass screening methods, UPLC-MS^E/TOF screening resulted in a 99% detection rate with expanded finding of drug metabolites.

Conclusion: Compared to nominal mass screening methods, UPLC-MS^E/TOF screening increases the detection of targeted drugs and their metabolites and, in addition, offered the potential for detection of non-targeted analytes via high resolution acquisition of molecular and fragment ion data.

Keywords: UPLC-MS^E/TOF Screening, Detection of Non-Targeted Analytes, Postmortem Drug Screening

S46

Synthetic and Analytical Challenges of Retigabine and *N*-Acetyl Retigabine

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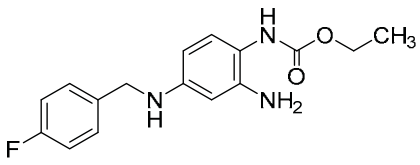
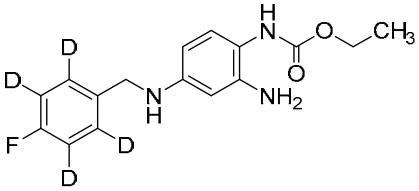
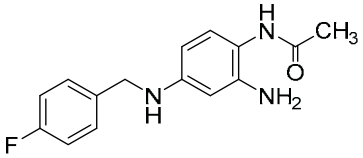
Introduction: Retigabine, or ezogabine, is an anticonvulsant used to treat partial seizures, and is marketed under the trade names Potiga© in the US and Trobalt© in the EU. Retigabine is metabolized in the liver to *N*-acetyl retigabine, among other metabolites. Both retigabine and *N*-acetyl retigabine are monitored in clinical and forensic applications and require certified reference materials (CRM's) for accurate quantitation.



Retigabine



Retigabine-D_4



N-Acetyl retigabine

Objective: High purity neat material of each of the above compounds was vital in producing the desired CRM's. The target compounds were purified or synthesized, certified, and then formulated into reference standards. Synthetic and analytical issues will be discussed in this presentation.

Method: The synthesis of retigabine-D_4 and *N*-acetyl retigabine required regio-selective approaches in order to prepare the desired carbamate or acetylated intermediates. The isolation, purification, and storage of intermediates and final products were affected by the sensitivity of this class of compounds to oxygen and light. Appropriate HPLC and GC conditions also had to be developed to account for the acid sensitivity and heat lability of these compounds which affected analysis.

Results: Synthetic and analytical challenges, as well as solution stability of these products were problematic prompting investigations into the most appropriate handling procedures to retain the integrity of these products. Retigabine-D_4 was synthesized after determining the best placement of the isotopic label based on LC/MS/MS transitions in order to deliver a stable internal standard.

Conclusion: Certified solution standards of *N*-acetyl retigabine, retigabine, and retigabine-D_4 were manufactured for use in clinical toxicology, forensic analysis and pharmaceutical research applications. Concerns regarding stability of the neat materials extended to solution stability, and both methanol and acetonitrile were evaluated for solubility and stability over a range of temperatures. Degradation as well as discoloration detected in methanolic solutions influenced the final formulation and storage of these standards.

Keywords: Retigabine, Ezogabine, Certified Reference Materials, Internal Standards, Forensic Analysis

S47
Enzymatic Hydrolysis Optimization for Urinary Cannabidiol

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Introduction: Non-psychoactive CBD has a wide range of therapeutic applications and may also influence psychotropic smoked cannabis' effects. Few methods exist for the quantification of CBD excretion in urine, and no data are available for phase II CBD metabolites.

Objective: To develop and validate a sensitive and specific quantitative assay for cannabidiol (CBD) to define its pharmacokinetics after controlled CBD administration, and to optimize hydrolysis conditions to obtain the highest urinary total CBD recovery.

Method: We optimized CBD-glucuronide and/or –sulfatehydrolysis, and developed and validated a GC-MS-EI method for urinary CBD quantification. We employed solid-phase extraction columns (Clean Screen THC - ZSTHC020) to isolate and concentrate analytes prior to GC-MS. Chromatographic separation was achieved within 6.5 min on a HP-5MS capillary column (30 m x 0.32 mm x 0.25 µm). The initial oven temperature of 160ºC, was increased at 20ºC/min to 250ºC and held for 2 min. Helium was the carrier gas at a constant flow of 1.5 mL/min and injection port temperature was 330ºC. Interface, ion source and quadrupole temperatures were 280ºC, 230ºC and 150ºC, respectively. We determined linearity, sensitivity, selectivity, extraction efficiency, intra- and inter-assay bias and imprecision, dilution integrity, stability, carryover, hydrolysis efficiency and recovery for the new assay. We evaluated temperature, pH, incubation time and amount of enzyme (*E. coli*, *H. pomatia* and Red Abalone) to maximize CBD concentrations.

Results: Linear least squares regression with $1/x^2$ weighting of calibration curves for non-hydrolyzed and enzyme hydrolyzed CBD samples had r^2>0.990. Linear ranges were 2.5–100 µg/L and 2.5–500 µg/L for non-hydrolyzed and hydrolyzed CBD samples, respectively. Similar bias (88.7–105.3%) and imprecision (1.4–6.4% CV) were achieved in both assays. Extraction efficiency of samples without hydrolysis (82.5–92.7%) was higher than for hydrolyzed samples (34.3–47.0%). A 1:2 (v/v) dilution yielded calculated concentrations 87.6–95.1% of target documenting dilution integrity. Hydrolysis efficiency was significantly affected by enzyme source, amount and incubation time. Optimal hydrolysis conditions were achieved with*E. coli* 5,000 units and overnight hydrolysis (16 h) at 37 °C; however, β-glucuronidase from Red Abalone (2,500 units) was selected over *E. coli*, due to a considerable difference in cost that outweighed the slight and non-significant increase in efficiency. This method was applied to urine samples collected in a study of CBD as a treatment intervention for opiate relapse. The Institutional Review Board of Mount Sinai Hospital approved the study and participants provided written informed consent. Urine specimens were collected at baseline and up to 8 h post-dose. Participants receiving two 400 mg CBD doses had non-hydrolyzed CBD concentrations <LOQ in all specimens. However, after CBD dosing and urine hydrolysis, concentrations ranged from 3.5–7825 µg/L after the first 400 mg dose, and 6.9–5214 µg/L after the second dose demonstrating the necessity of hydrolysis.

Conclusion: This is the first method to evaluate CBD hydrolysis and quantification in urine.This method will be useful to determine CBD pharmacokinetics and free/glucuronide ratios after oral CBD or cannabis-based medicinal extract administration.

Keywords: Cannabidiol, Cannabidiol-Glucuronide, Enzymatic Hydrolysis, Urine Cannabinoids

Supported by the National Institutes of Health, Intramural Research Program, NIDA, NIDA grants DA027781 and UL1RR029887 and CAPES (Brazil).

S48

Validation of the Randox Drugs of Abuse V Biochip Array Technology for Detection and Semi-Quantification of Synthetic Cannabinoids in Urine

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Introduction: Synthetic cannabinoids (SC) are designer drugs whose prevalence has rapidly increased in Europe and the US. SC do not produce positive results on traditional cannabinoids immunoassays making it difficult to perform high volume testing. To try to meet this type of testing, Randox offered the first commercially available assay for synthetic cannabinoids in urine.

Objective: The purpose of this study was to evaluate the Randox biochip array technology for SC in urine, and its applicability in a high throughput workplace drug testing forensic laboratory.

Method: The SC biochip has 4 different SC antibodies; SC-I, SC-II, and SC-III target JWH-018 (100% cross-reactivity), while SC-IV targets JWH-250. The kit included 9 calibrators and 2 quality control samples; the Evidence® high-throughput analyzer was utilized for all analyses. Limits of detection (LOD), linearity, imprecision, %bias, selected cutoff concentrations and samples ±25% and ±50% of cutoffs were evaluated. Older and newer versions of the Randox SC biochip assay were evaluated; these contained the same antibodies but calibrators were prepared in a different base, thus, interference, carryover, and cross-reactivity were assessed only with the older kit. 20,017 urine specimens were analyzed with results confirmed by LCMS/MS to determine diagnostic sensitivity, specificity and efficiency.

Results: LODs for SC-I, SC-II, SC-III, and SC-IV (older and newer) were 5, 11, 3, and 2µg/L based on our authentic negative urine samples (n=10), respectively. Linearity (0–225µg/L SC-I-III, 0-150µg/L SC-IV) was obtained (R^2>0.995), although mean %bias was -62.9 to -47.3%. Intra- and inter-assay imprecision are summarized below:

	Imprecision(%CV)				**Mean %Bias**			
Level(µg/L)	Intra (n=4)		Inter(n=20)		Intra (n=4)		Inter (n=20)	
	Older	Newer	Older	Newer	Older	Newer	Older	Newer
15 (SC I-IV)	9.2	8.4	16.1	13.7	-56.7	-31.7	-54.9	-31.8
30 (SC I-IV)	7.6	4.5	21.4	14.0	-60.7	-44.9	-57.0	-39.3
60 (SC IV)	6.5	8.0	23.2	11.5	-44.8	-39.2	-43.7	-33.1
90 (SC I-III)	6.8	10.2	35.5	32.2	-69.6	-50.6	-61.5	-45.8

Samples fortified at+50% of LOD could not be differentiated from a negative sample. %bias and imprecision in SC-I, -II, -III made separation of concentrations around the cutoffs difficult to distinguish if there was less than a 10µg/L concentration difference. There was no carryover (>LOD) in samples preceded by 500µg/L JWH-018 or JWH-250. Interference results were <LOD for all SC. Negative urine samples fortified with JWH-018 (100µg/L) or JWH-250 (100µg/L) and adulterated with bleach or hydrogen peroxide produced 10-15% increases in concentration from non-adulterated samples. SC biochips cross-reacted with 23 SC parent and 37 metabolites. No cross-reactivity was detected for XLR-11 and UR-144. 1,428 (7.1%) authentic urine specimens screened ≥Randox recommended cutoffs, only 285 (20.0%) confirmed positive by LCMSMS for one or more SC metabolites. Six presumptive negative specimens confirmed positive. Diagnostic sensitivity, specificity, and efficiency for the SC biochip were 97.9%, 48.3%, and 54.1%, respectively.

Conclusion: In validating performance of the Randox DOA V SC biochip assay, we found the inaccuracy (%bias) greatly affected performance, including imprecision. More than 20 SC and metabolites cross-reacted in the assay. Ninety samples can be screened for up to 11 drug classes with the DOA V biochip on the Evidence analyzer in 1–1.5 h in 350–500 µL urine. Although sensitivity was high, specificity was only 48.3% based on LCMS/MS confirmation.

Keywords: Biochip Array Technology, Synthetic Cannabinoids, Immunoassay, Urine

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

S49

Development and Evaluation of a High-Throughput Screening Method for Synthetic Cannabinoids/Metabolites in Urine with Q-TOFMass Spectrometer and Multiplexed LCs

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Introduction: Chemically engineered to mimic Δ9-tetrahydrocannabinol (THC) pharmacological activity, synthetic cannabinoids are a major health problem. A rapid, sensitive screening test for synthetic cannabinoids/metabolites is needed. Immunoassays are available, but lack flexibility for adding new analytes as market changes constantly occur. Liquid chromatography tandem mass spectrometry (LC-MS/MS) offers increased sensitivity and specificity for identifying synthetic cannabinoids/metabolites and ability to add newly emerging analytes. TripleTOF® 4600/5600 high resolution accurate mass systems are capable of full MS range data collection and simultaneous MS/MS confirmation, providing an ideal platform for screening specified targets and unregulated new analogues. Recent developments in LC multiplexing further improves throughput and makes LC-MS/MS effective for screening applications.

Objective: To develop and validate an LC-MS/MS-based high throughput screening (HTS) method for synthetic cannabinoids/metabolites on the AB SCIEX TripleTOF® 4600/5600 mass spectrometers and MPX™-2 High-Throughput System. Our goal was to optimize LC conditions to minimize matrix effects, improve data quality, and achieve injection-to-injection cycle time around 2 min to match or exceed immunoassay screening throughput. Further, it is preferred that positive screening results can be confirmed simultaneously with multiple screening criteria including MS/MS matching.

Method: Target synthetic cannabinoids/metabolites included JWH-018, JWH-019, JWH-073, JWH-081, JWH-122, JWH-200, JWH-210, JWH-250, JWH-398, RCS-4, AM-2201, MAM-2201, UR-144 and their metabolites and JWH-203, AM-694, RCS-8 and XLR-11 parent compounds. Urine samples were mixed with internal standards, diluted in 50% methanol/water and centrifuged to remove particulates. Ten µL supernatant was directly injected for LC-MS/MS analysis on TripleTOF®5600 mass spectrometer, or the TripleTOF® 4600 system that was coupled to an MPX™-2 system. HPLC separation was performed by gradient elution on a Restek Ultra Biphenyl column(50 × 2.1 mm, 5 µm) at 30°C with 5 mM ammonium formate in water and methanol mobile phases. Positive mode electrospray ionization-MS analysis was performed on the TripleTOF system acquiring full scans followed by information-dependent data acquisition MS/MS scans with 12 maximum candidate ions with or without inclusion lists.

Results: The fast scan-speed of TripleTOF®4600/5600 LC/MS/MS Systems permits a TOF-MS survey scan followed by 12 information-dependent MS/MS scans with a cycle time of ~0.5 sec, permitting retrospective data mining and eliminating sample reinjection. An individual data file was acquired for each sample. Data processing was based on a targeted approach with extraction ion list. Screening results were determined with multiple criteria including mass accuracy, retention time, isotopic ratio and MS/MS matching. MS/MS library matching significantly improved screen quality by minimizing false positive/negative rates achieving 0.4-2 µg/L limits of detection with -16 to -47% matrix effects (n=7).A two-fold increase in throughput was achieved with MPX-2, with a final injection-to-injection cycle time of 2.09 min.

Conclusion: A novel HTS method for synthetic cannabinoids/metabolites wit**h** TripleTOF®and MPX™-2 system was developed and evaluated. Workflow was simple and fast, while offering flexibility for adding new compounds, with higher sensitivity and specificity than immunoassays. This approach could be easily utilized for screening other drug classes, such as synthetic cathinones.

Keywords: Synthetic Cannabinoids, Triple-TOF, High-Throughput Screening

Research funding by AB SCIEX and the National Institutes of Health, IRP, National Institute on Drug Abuse.

S50

Drug Screening in Urine Using Multiple Methods (Immunoassay, GC-MS, LC-MS/MS): Is There an Easier Way?

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Introduction: In the forensic laboratory, drug screening is done by a combination of methodologies that may include 2 or more of the following: immunoassay (IA), ELISA, GC-MS or LC-MS/MS. Recently, drug screening methods that utilize high resolution mass spectrometry (HRMS) have been published in the literature. Despite the interest in these approaches, few studies have compared HRMS screening to traditional drug screening methodologies to determine how HRMS might compliment or replace traditional methods.

Objective: The objective of this study was to develop LC-HRMS and LC-MS/MS broad spectrum drug screening methods that are identical except for the mass spectrometer used and to compare the two methods and IA screening for their ability to identify drugs and metabolites in routine specimens.

Method: For both MS methods, urine samples were diluted 1:10 and separation was performed using a Phenomenex Kinetex C18 column (50x3.00mm, 2.6µm) with a 10 minute gradient from 2%-100% organic (MPA – 5mM ammonium formate, 0.05% formic acid and MPB – 50:50 methanol:acetonitrile, 0.05% formic acid). For the MS/MS method, 230 drugs and metabolites were monitored in positive-ion MRM-IDA-EPI mode with an ABSciex QTRAP®3200. The HRMS method was developed with an ABSciex TripleTOF®5600 operating in positive-ion HRMS full scan mode with IDA triggered acquisition of HRMS product ion spectra. Data analysis for both methods was done using PeakView® and LibraryView™ (AB Sciex). The same spectral library, containing spectra generated from both instruments was used for data analysis. Immunoassay screening for opiates, oxycodone, EDDP, benzoylecgonine, amphetamines and benzodiazepines was performed on an Advia 1800 (Siemens) using Microgenics immunoassays (Thermo Scientific).

Results: For the common drug/drug classes (listed above), immunoassay screening results were compared to the LC-MS/MS and LC-HRMS results for 100 urine samples sent for routine analysis. The overall sensitivities of the methodologies for the drug/drug classes were 89% (IA), 96% (MS/MS) and 89% (HRMS). The overall specificities were 94% (IA), 99% (MS/MS) and 100% (HRMS). Using oxycodone as an example drug, the sensitivities were 91% (IA), 95% (LC-MS/MS) and 91% (LC-HRMS) and the specificities were 95% (IA) and 100% (MS/MS and HRMS). When comparing the methods for their ability to detect cocaine-benzoylecgonine the sensitivities were 85% (IA), 98% (MS/MS and HRMS) and the specificities were all 100%. The LC-MS/MS and LC-HRMS methods also detected additional drugs for which immunoassays are not available. In the 100 samples, the MS/MS targeted method detected 614 total compounds of which 92% were confirmed positive by a second method. Using targeted analysis, the HRMS method detected 430 total compounds of which 98% were confirmed positive. For both MS methods, the LLOD s ranged from 0.5-50ng/mL. In general, the LLODs were lower for the MS/MS method compared to the HRMS method, which may explain way more compounds were detected using the MS/MS method in the 100 urine samples. Comparison of these methods for analysis in other matrices is underway.

Conclusion: The MS methods were more sensitive and specific compared to IA screening. They were also capable of detecting additional compounds with a high degree of confidence in positive identifications.

Keywords: High Resolution Mass Spectrometry, Drug Screening, Method Comparison

S51
Development of a High Resolution Mass Spectrometry Method for Designer Drugs

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Introduction: Designer drugs have been a topic of much discussion since their popularity as a legal high started in 2004 in Europe. To date, several states have moved to ban the use and sell of synthetic cannabinoids, cathinones, and piperazines. However, most state and federal law makers prefer to ban specific compounds, not structural moieties. Even with state and federal analog laws, the onus to prove similar pharmacology and pharmacokinetics has made prosecution difficult. As such, the detection of these designer drugs has always been mostly reactive. Laboratories who generally rely on screen-confirm methodologies for their testing were further hampered until immunoassays for these compounds were developed. To date, most of the immunoassays developed are limited and some are already outdated. Moreover, users rarely know what drugs they are taking; therefore confirming for "spice" or "bath salts" can mean many classes of compounds-some legal and some illegal.

Objective: In order to remain current with the compounds on the market, based on seizures and blogs, a drug monitoring method using liquid chromatography-online sample extraction- tandem mass spectrometry with full scan orbitrap was developed. This method allows for targeted analysis as well as unknown compound elucidation. Testing, in both urine and saliva, for several designer drugs including synthetic cannabinoids, synthetic cathinones, and piperazines, as well as amphetamines and benzodiazepines, was accomplished with this method.

Method: The method quantifies 63 targeted compounds as well as detects untargeted compounds. The targeted compounds are amphetamine, methamphetamine, phentermine, MDMA, MDA, MDEA, alprazolam, α-hydroxyalprazolam, diazepam, flunitrazepam, nordiazepam, oxazepam, temazepam, 7-aminoclonazepam, PCP, cocaine, BZE, TFMPP, mCPP, BZP, methylone, α-PVP, pentedrone, methylhexanamine, 4-MEC, ethylone, pentylone, butylone, MDPV, mephedrone, MAM2201, RCS-8, RCS-4, JWH398, JWH018, AM2201, HU210, JWH073, JWH200, AM694, AKB48, JZL184, JZL195, STS135, UR144, UR144 N-pentyl metabolite, URB447, URB597, URB602, URB754, URB937, XLR11, JWH018 pentanoic acid metabolite, JWH073 butanoic acid metabolite, AM2201 N-hydroxypentyl metabolite, JWH 018 Ω and Ω-1 pentyl metabolites, JWH 019 6-hydroxyhexyl metabolite, JWH022, JWH081 N-hydroxypentyl metabolite, JWH122 N-hydroxypentyl metabolite, and JWH210 N-carboxyl metabolite.

Urine samples are hydrolyzed, spiked with internal standards, and injected on the turbulent flow column. Saliva samples are spiked with internal standards, filtered, and injected on to the turbulent flow column. Standards are purchased from Cayman Chemical and Cerilliant. Mobile phase is water and methanol with ammonium formate and ammonium acetate additives. All samples were run on a Transcend TLX-2 (Thermo Scientific) coupled to an Exactive Orbitrap (Thermo Scientific). Run time for the method is under 8 minutes.

This method is able to test in concentration range of 100pg/mL-1000ng/mL with a LLOD below 100pg/mL and a LLOQ at 100pg/mL. The method is linear in the aforementioned quantification range. The method was tested for matrix suppression and enhancement and none was seen in the quantification window as defined as ±25%. Imprecision and inaccuracy have a specification limit of ±20% for all compounds; however repeated injections are able to perform better than ±10%.

Results: Detection of compounds in each class of drugs was found. Often bath salts confirmations contain amphetamines, cocaine, and/or benzodiazepines. Specifically, "bath salt" positives were found for methylhexanamine and α-PVP. However, most of the confirmation samples did not contain a known synthetic cathinone. More than 50% of the samples were positive for methamphetamine and amphetamine, alprazolam or diazepam and their respective metabolites, or cocaine and BZE.

Similarly, "spice" confirmations rarely contained synthetic cannabinoids. Instead positives were detected for THC and THC metabolites, nicotine metabolites, and other depressants (e.g. opioid-receptor agonists). FAAH and MAGL mediators, however, were most often positive in the designer drug confirmation samples for "spice".

Conclusion: Ultimately, a drug monitoring method was developed to allow for detection of designer drugs to help in the fight against the use of these compounds. Specifically, it also allows for reporting of compounds that are not generally categorized as "spice" or "bath salts" compounds. With this type of methodology, laboratories can now be more proactive in their testing by detecting targeted and non-targeted analytes.

Keywords: Designer Drug Detection, Drug Monitoring Method, Detection of Non-Targeted Analytes

S52

Pitfalls of Analyzing Urine Specimens for the Presence of Cycloalkyl Functionalized Indoles: XLR-11

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Introduction: After the federal ban (June 2012) on phenyl acetyl and naphthyl indoles, producers of designer drugs turned to cycloalkyl functionalized indoles, including UR-144 and XLR-11 (5-fluoro UR-144) that were not included in the legislation. At RTL, our synthetic cannabinoid urine screen now tests for the metabolites and parents of 19 drugs including XLR-11 and UR-144. The detection of XLR-11 in urine is complicated by the lack of standards for the prominent metabolites and instability of the parent drug when heated. For example, the prominent XLR-11 oxidation metabolite found in urine specimens fails to give a spectral or retention time match with the XLR-11 N-(4-hydroxypentyl) analytical standard—the only standard currently available. A prominent degradant of XLR-11, that is formed upon pyrolysis, has been identified as the 3,3,4-Trimethylpentenoyl isomer and is commercially available. To determine the differences in the identities of the phase I metabolites of XLR-11 and XLR-11 degradant, both were separately incubated with human liver microsomes and the resulting solutions were analyzed by LC-MS/MS. The results of these analyses and findings from our synthetic cannabinoid screen will be discussed.

Objective: To determine the differences in the identities of the phase I metabolites of XLR-11 and XLR-11 degradant.

Method: Standard reference material for XLR-11 and XLR-11 degradant were obtained from Cayman Chemicals. Both compounds were incubated separately at a concentration of 2 μM for 0, 15, 30 and 60 minutes with pooled human liver microsomes S9 fraction (Molecular Toxicology, Boone, NC) in pH 7.4 phosphate buffer and the necessary NADPH regenerating solutions. The reaction was stopped by removing 500 L of the solution into a centrifuge tube on wet ice and adding 500 L of acetonitrile. After centrifugation, a 20 L aliquot of the supernatant was analyzed by LC-MS/MS in MRM or full scan mode using the conditions previously reported by Rana et. al (SOFT 2010, abstract # S51).

Results: Only trace amounts of the 4-hydroxypentyl metabolite of XLR-11 were present in the microsome incubation samples though isomers were recorded at different retention times and with different product ion spectra. These results agreed well with positive urine specimens. UR-144 hydroxypentyl and pentanoic acid metabolites were identified in the microsome incubations of XLR-11, and their spectra and retention times agreed with those of their respective standards. For the XLR-11 pyrolysis product, isomers of the UR-144 hydroxypentyl and XLR-11 hydroxypentyl metabolites were identified. The spectrum and retention time of the predominant oxidation in the microsomal incubation of the XLR-11 pyrolysis product matched those of the predominant oxidation observed in the urine specimens. Parent XLR-11 was observed in less than 2% of the positive samples. XLR-11 pyrolysis product also excreted in urine unchanged in a similar percentage of specimens and was observed at an earlier retention time than XLR-11.

Conclusion: Monitoring urine for the pyrolysis products of XLR-11 and its metabolites is recommended to avoid false negative results. Further research on the application of the pyrolysis products of XLR-11 and their metabolites for urine testing is needed.

Keywords: Synthetic Cannabinoids, Microsome Incubation, XLR-11, UR-144

S53

Validation of the Only Commercially Available Immunoassay for Synthetic Cathinones in Urine: Randox Drugs of Abuse V Biochip Array Technology

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Introduction: Efforts to deter abuse of synthetic cathinones, "bath salts," is hampered by the lack of a cost effective immunoassay for initial specimen screening. Recently, Randox developed an immunoassay for drugs of abuse detection: the Randox Drugs of Abuse V (DOA-V) biochip. The chemically modified biochip surface contains 11 drug testing regions, two specifically for synthetic cathinones. Bath Salt I (BSI) targets mephedrone/methcathinone, and Bath Salt II (BSII), 3',4'-methylenedioxypyrovalerone (MDPV)/ 3',4'-methylenedioxy-α-pyrrolidinobutiophenone (MDPBP). Anonymized urine specimens were screened utilizing the Randox DOA-V biochip assay to determine bath salt prevalence. After specimen analysis, Randox released a new kit with the same antibodies but with altered linearity, and reconstitution of calibrators and controls in lyophilized urine, instead of deionized water (older kit).

Objective: To evaluate the feasibility of the Randox DOA-V biochip assay for high throughput screening for synthetic cathinones in urine.

Method: Validation studies were performed with the older and newer DOA-V synthetic cathinones kits and the fully automated Evidence® Analyzer (Model No. EV180). Randox designated linear ranges were 0-20.7µg/L (BSI) and 0-1053µg/L (BSII) for the older kit, and 0-32.2µg/L (BSI) and 0-1037µg/L (BSII) for the new kit. Validation of the synthetic cathinones BSI and BSII included limits of detection (LOD), linearity, bias, imprecision, and proposed Randox cutoffs of 5 & 30µg/L for BSI and BSII, respectively. Cross-reactivity, interferences, and carryover were assessed for the Randox DOA-V older kit only, as antibodies did not change.

Results: LODs were defined as mean observed concentrations of drug negative urine samples + 3SD (n=60). LODs for older and new kits were 0.35 (BSI), 8.5 (BSII), 0.18 (BSI) and 9.2 µg/L (BSII), respectively. No carryover was observed for BSI and BSII. Linearity was acceptable (R^2>0.9999) for both BSI and BSII DOA-V kits. Acceptable bias (within ±20%) was achieved for BSI (mephedrone) across the linear range for the older kit; however, %bias for the new kit was not (-53 to -38%). For BSII (MDPV), %bias was outside acceptable ranges for both older (mean %bias: -37%) and new (mean %bias: -39%) kits. The assay demonstrated acceptable intra-assay imprecision (n=4) for detection of mephedrone and MDPV over three concentrations (<20% CV). Inter-assay (n=20) and total imprecision (n=5) varied across all mephedrone concentrations (18-42%) and was <22% for MDPV concentrations. Cutoff values were difficult to evaluate due to consistently lower concentrations than target. Cross-reactivities to mephedrone were similar to the reported Randox cross-reactivities, with additional synthetic cathinones reacting to BSI. MDPV cross-reactivities were lower than the expected Randox values, but consistent with the under recovery observed for BSII in this study. L-ascorbic acid, hemoglobin, acidic urinary pH and oxidizing agents produced interferences.

Conclusion: Although %bias for the detection of BSI (mephedrone) and BSII (MDPV) was not within acceptable limits for the new kit, the Randox DOA-V biochip array remains the only immunoassay screening kit available for preliminary detection of synthetic cathinones in urine.

Keywords: Synthetic Cathinones, Bath Salts, Urine, MDPV, Mephedrone

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Abstracts Of Poster Presentations

P01

On-Line SPE LC-MS/MS Drug Screening in Serum on a Hybrid Quadrupole -Linear Ion Trap Instrument

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Introduction: For research only, not for use in diagnostic procedures. Comprehensive screening for the detection of drugs and toxic compounds in biological samples is an important function of toxicological analysis. As the demand to monitor the ever increasing number of drugs continues to rise, so too does the need to detect and quantify these compounds in a simple, single and automated run; providing a fast turn-around time for the results. This presentation describes the rapid cleanup of serum samples using On-line Solid Phase Extraction (SPE) LC-MS/MS screening and confirmation. Combining Multiple Reaction Monitoring (MRM) and product ion spectral acquisition allows for compound identification with highest confidence based on mass spectral library matching. The automated workflow monitors large panels of analytes; detecting and quantifying these compounds in a single run.

Objective: Develop a comprehensive serum drug screening and confirmation method using On-Line SPE-LC/MS/MS

Method: Serum was spiked with drug mixture stock solutions (containing 130 drugs) making concentrations ranging from 0.1 to 2000 ng/mL to prepare the calibrators; 10, 1000 ng/mL for QCs. One mL of serum was acidified with formic acid and 100 µL of internal standard solution added. On-line SPE and elution was performed using a Spark Holland Symbiosi Pico System with HySphere Resin GP 10 cartridges. LC separation was performed on a Phenomenex Kinetex 2.6 C18, 100 Å, 50 x 3.00 mm column at 40 °C. A 15 minute gradient of water and methanol with ammonium formate buffer was employed at 0.4 mL/min flow rate; 10 µL injection volume. The MS was operated in positive MRM mode. Dependent product ion spectra were acquired after being triggered from MRM Information Dependent Acquisition (IDA) survey scans.

Results: Up front cleanup of biological samples increases assay performance and reduction of matrix effects in LC-MS/MS analysis. Extraction methodologies used, however, are often manually performed making sample processing times and cost of analysis unnecessarily high and can be prone to human error effecting precision, accuracy and recoveries. SPE is a well accepted sample preparation technique and provides advantages in selectivity and produces cleaner extracts over liquid/liquid extraction and is also more amenable for automation making the approach ideal for high throughput comprehensive drug screening.
Using a hybrid linear ion trap allowed the generation of enhanced product ion spectra that contain information of the complete molecular fingerprint of compounds that were searched against mass spectral libraries significantly reducing the risk of false positive results. This technology combined with the automated On-line SPE sample clean-up made it possible to monitor extremely large panels of analytes within a very short time with high confidence in identification. The method was successfully applied to quantify and identify drug compounds from spiked serum samples.
The automated On-Line SPE LC/MS/MS workflow provides rapid extractions, high recoveries, and minimized matrix interferences with complete automation capabilities towards high throughput chromatographic analysis of 130 drugs. Linearity was achieved between 1-1000 ng/mL for most drugs and typical recoveries were greater than 80%. Matrix effects were evaluated at 10 ng/mL concentrations, using one lot of serum, and % accuracy differences were typically less than 20%. Table 1 shows some specific examples with % CV and % Accuracy values at the LOQ.

Analyte	%CV at 1ng/ mL LOQ (n=3)	%Accuracy	Matrix Effect (% Accuracy Difference)	Recovery (%)
Acetominophen	0.9	102	15	98
Beclometasone	8.5	109	6	61
Bezafibrate	20	80	23	55
Clonazepam	20	112	4	112
Nalorphine	9	87	1	81
Norfentanyl	5	99	3	14
Oxycodone	3	97	11	86
Propanolol	6	90	49	61
Sotalol	2	103	15	97
Terfenadine	5	87	16	76

Conclusion: Analysis for large panel of drugs with automated sample preparation, detection and confident identification in a single injection was achieved.

Keywords: On-Line SPE-LC/MS/MS, Automated Sample Preparation, Comprehensive Serum Drug Screen

P02

A Highly Selective, Robust and Simple Drug Screening Method Using an Accurate Mass Quadrupole/Time of Flight Tandem Mass Spectrometer

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Introduction: Forensic laboratories performing drug screening analyses wish to identify as many compounds as possible from a single experiment, and desire the capability and flexibility to perform retrospective data analysis for the purpose of identifying compounds that were not included in the initial targeted screen. Recently, high-resolution/accurate-mass mass spectrometers (MS) have gained popularity as a tool for drug screening, largely due to the ability to run simple and generic methods, and the possibility of performing retrospective data analysis. However, accurate mass measurements alone on a Liquid Chromatography-MS system cannot resolve all potential interferences; therefore we have increased the analytical selectivity by developing a rapid LC-MS/MS method using a hybrid Quadrupole/Time of Flight (QTOF) tandem mass spectrometer. We also employed a multiplexed LC system, to increase throughput by staggering sample injections on two separate LC streams.

Objective: Develop a selective, robust accurate mass drug screening method.

Method: To minimize sample preparation, we have employed a "dilute and shoot" approach. The chromatographic run time was set to less than 7 minutes; however the sample-to-sample injection time on the multiplex LC system was less than 4 minutes. In the LC-MS/MS method developed here, we acquired both accurate mass single TOF-MS scans, and scheduled product ion scans (MS/MS), for 41 target pain panel analytes in urine matrix. Data reduction software automatically performed library-searching of the experimental data against a high-resolution / accurate-mass MS/MS library.

Results: It was found that all compounds examined in this analysis were correctly identified. No false positives were observed in urine matrix, due to the added selectivity provided by MS/MS measurements, and by library searching of experimental MS/MS spectra against a database containing accurate-mass MS/MS spectra. In contrast, when the compound identification relied solely upon the accurate-mass single MS information, false positives were observed in several instances. In spiked urine, 4 out of 15 compounds displayed interferences in the retention time window. Chromatographic separation is absolutely essential if TOF-MS alone is being used. Using TOF-MS/MS all interferences were removed from the spectra of the 4 compounds. For unambiguous identification MS/MS is required, through MS/MS extracted ion chromatograms or through library searching. The use of a multiplexed LC system enabled an increase in sample throughput of almost 2-fold.

Conclusion: By taking advantage of both MS and MS/MS scanning in a single method on the hybrid Quadrupole/Time of Flight instrument, this 'no-compromises' approach enabled superior targeted and non-targeted analysis on an accurate-mass LC-MS/MS system. The use of a multiplexed LC system enabled high throughput analysis.

Keywords: Mass Quadrupole/TOF-MS, Mass Drug Screening

P03

Targeted LC-MS/MS Screening of Urine Toxicology Samples Using Advanced Processing Software Tool

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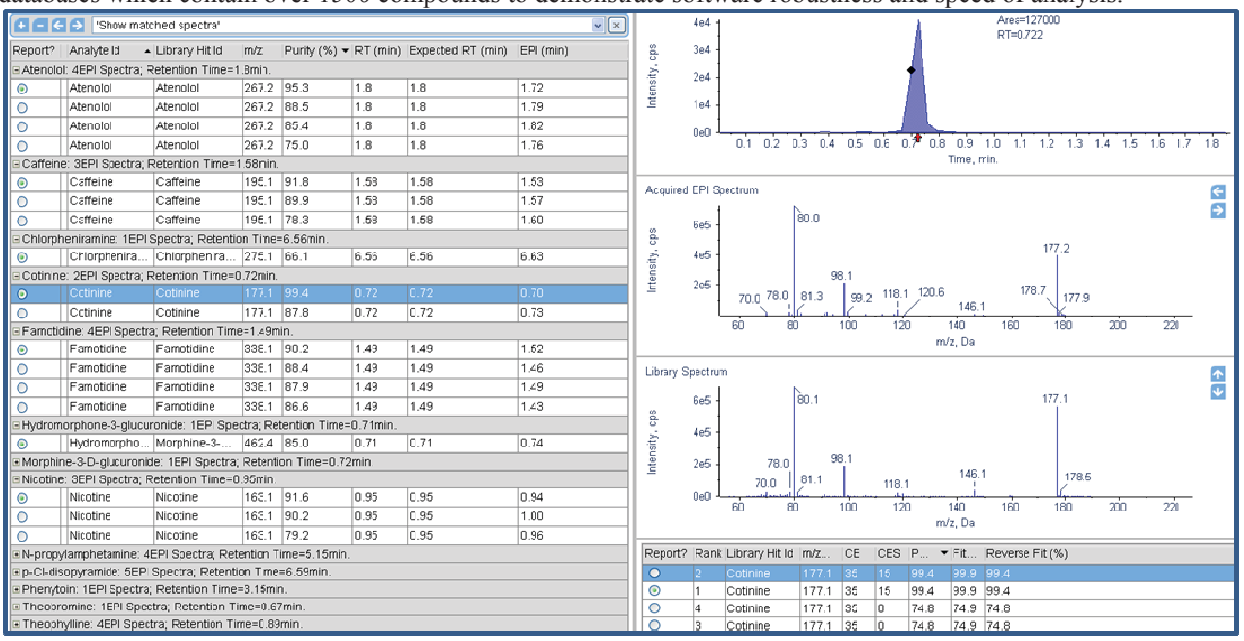
Introduction: LC/MS/MS analysis combined with automated library searching for compound identification has emerged as an approach of choice to perform both screening and confirmation in forensic analysis. To meet the needs of laboratories performing analyses of large panels of compounds, a complete and simple software solution is required, offering an automated workflow which includes data collection, processing, as well as revision and reporting. In this study we present the use of an elegant software tool which provides an access to interactive MS/MS library searching results.

Objective: The objective of this study was to demonstrate that the combined use of "traditional" detection via LC/MS/MS and confirmatory MS/MS library searching generates the most reliable results, and avoids the possibility of reporting false positives.

Method: A set of selected urine toxicology samples was analyzed for a large panel of drugs of abuse. Applied sample preparation procedure included centrifugation of a urine aliquot mixed with the internal standard, followed by additional centrifugation, final dilution and LC/MS/MS analysis. Data was acquired utilizing an AB SCIEX 3200QTRAP® mass spectrometer, coupled with an Agilent 1200 LC system. The mass spectrometric portion of the acquisition method consisted of MRM detection of the target analytes, using *Scheduled* MRM Pro algorithm, followed by two dependent Enhanced Product Ion Scan (EPI) experiments to collect the confirmatory information. The EPI experiments were performed using the Linear Ion Trap of the specified LC/MS/MS systems. The confirmatory EPI spectra were collected utilizing the Collision Energy Spread (CES) option to obtain the largest possible coverage of the fragment ions characteristic for low-, medium- and high-collision energy regimes. These spectra were searched against a spectral library containing more than 1500 compounds, including pain panels, drugs of abuse and their metabolites. The interpretation and verification of compound- and sample-specific data was performed using a prototype *Interactive Data View* (*IDV*) tool before generating the final reports.

Results: Utilization of the new software solution enables automation of the library searching for the confirmatory EPIs in the context of the detected analytes (integrated chromatographic peaks) and specified MRM detection limits. It is possible to review both quantitation and screening results before generation of the final reports as well as dynamically adjust the acquired EPIs to be included into the report (see table below, "Cotinine" example). The results can be queried for a batch or for individual samples. The *IDV* performance was evaluated against the databases which contain over 1500 compounds to demonstrate software robustness and speed of analysis.



Conclusion: The software solution presented here integrates quantitation of the target analytes with the confirmatory MS/MS library searching to produce a detailed summary of the processed data for revision. The tool is demonstrated to enable updating the library search parameters and dynamic selection of the acquired and library full scan MS/MS spectra to be included into the final reports.

Keywords: Analyses of Large Panels, AB SCIEX 3200QTRAP®, LC/MS/MS

P04

The Determination of Cathinones and Their Metabolites in Urine Samples

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Introduction: In recent years, cathinones have been abused seriously worldwide. The structures of cathinones are closely related to amphetamines, which have similar stimulating effects and are somehow better investigated. These compounds have been marketed over the internet or in so-called head shops as "legal highs" and have gained popularity among drug users.

Objective: For detection and determination of 18 designer cathinones and their metabolites in urine samples, gas chromatography-tandem mass spectrometry (GC-MS-MS) and gas chromatography mass spectrometry (GC-MS) methods were developed requiring only 1 mL of urine.

Method: The urine was extracted by solid phase extraction (SPE) with Bond Elut certify 130 mg 3 mL column, using deuterium labeled methylone, mephedrone, methdrone, 4-fluoromethcathinone, methylenedioxypyrovalerone (MDPV) and diethylcathinone as the internal standards.

The technological improvements of the present study on cathinones include: (1) The detection limits of GC-MS and GC-MS-MS of MS2 to screen the most commonly prescribed 20 cathinones and their metabolites were less than 200 ng/mL. (2) 18 cathinones and their metabolites were simultaneously quantified by multiple reactions monitoring mode of GC-MS-MS.

Results: It was shown that the limit of quantitation range was 1~5 ng/mL, determination coefficient range was 0.990~0.999, and the inter-day precision range was 5% ~20%. (3) Detection of 60 urine specimens collected from court cases helped procure perspective on the situation of cathinones abuse in Taiwan .

Conclusion: The aim of this paper is to summarize the information about cathinones abuse in Taiwan. Furthermore the paper could also be applied to the determination of cathinones abuse in specific cases. In the further, we will develop a visible method for determination of cathinones in hair samples.

Keywords: Cathinone, MDPV, GC/MS/MS, Metabolite

P05

Comprehensive Toxicological Screening Using Generic MS/MS^{ALL} Acquisition on a Q-TOF Tandem Mass Spectrometer

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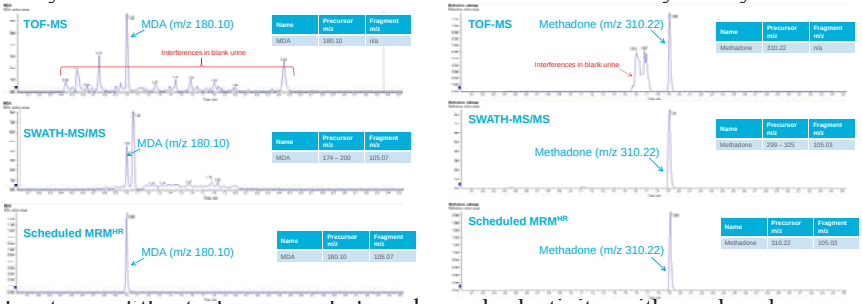
Introduction: For research use only, not for use in diagnostic procedures. Advances in Time-of-Flight (TOF) instrumentation have yielded analytical systems with the speed, sensitivity and dynamic range to be useful for rapid toxicology screening when coupled to liquid chromatography (LC). Q-TOF tandem mass spectrometer functionality enables a more selective analysis of compounds, by leveraging the enhanced specificity of LC-TOF-MS/MS measurements. However, compounds of interest must be pre-selected for isolation and fragmentation, therefore a targeted MS/MS approach does not allow performing retrospective MS/MS data analysis to identify previously unknown compounds. In this work we present a novel approach that provides both the enhanced selectivity of MS/MS measurements *and* the ability to perform retrospective data analysis. The methodology employs Sequential Windowed Acquisition of all Theoretical (SWATH) MS/MS across a mass range of interest.

Objective: To explore the utility of a novel experimental technique providing high-resolution MS/MS^{ALL} data, by employing sequential windowed acquisition on a Q-TOF instrument. We aim to demonstrate that the application of this generic, non-targeted methodology enables truly comprehensive toxicological screening, since the MS/MS data may be re-interrogated at any time, for any number of target compounds or previously unanticipated compounds.

Method: Blank urine was spiked with known concentrations of common drugs of abuse ranging from 50% cut-off levels to 50x cut-off levels, and high-resolution accurate mass MS/MS^{ALL} data was collected using Sequential Windowed Acquistion of all Theoretical (SWATH) MS/MS. The Q1 acquisition window for each SWATH scan was 15 amu, and the method covered a mass range from m/z 125 to 500. Extracted ion chromatograms were monitored for the characteristic MS/MS fragment ions for 41 compounds of interest. To evaluate the performance of the SWATH method versus conventional analytical approaches, the same spiked urine samples were analyzed using (i) a TOF-MS survey scan with Information-Dependent-Acquisition (IDA) triggering of up to 20 product ion scans, and (ii) a dedicated, looped MS/MS experiment, targeting each of the 41 compounds of interest.

Results: A comparison of MS/MS^{ALL} with sequential windowed acquisition versus (i) TOF-MS, and (ii) targeted TOF-MS/MS was performed. Using only TOF-MS, even with a small extraction window (0.010 Da), we have observed the possibility of interferences. In spiked urine, 5 out of 15 compounds – Methadone, MDA, Hydromorphone, Norpropoxyphene, and Norfentanyl – displayed interferences in the retention time window of the target compound. Therefore, chromatographic separation was absolutely essential if TOF-MS alone was employed. Using TOF-MS/MS all interferences were removed from the extracted ion chromatograms of the 5 compounds. We therefore conclude that for unambiguous identification, MS/MS measurements are required.
Using MS/MS^{ALL} with SWATH acquisition, it was possible to extract XICs for fragment ions of all 41 target compounds, as well as any previously unanticipated compounds. The XICs from the SWATH data demonstrated far superior selectivity compared to TOF-MS data, but less selectivity than targeted TOF-MS/MS for certain analytes. Nevertheless, this approach enabled retrospective data analysis of MS/MS data, which could not be achieved using a targeted TOF-MS/MS experimental approach. Performing MS/MS^{ALL} with SWATH acquisition provides the enhanced selectivity of MS/MS measurements, while simultaneously enabling retrospective data processing due to the non-targeted nature of the experimental approach. Although the selectivity is reduced compared to targeted MS/MS due to the larger Q1 isolation window (15 amu in the work presented here), the size of the Q1 isolation window can be adjusted by the user to obtain the desired balance between selectivity and cycle time.



Conclusion: Major advantages of this technique include: enhanced selectivity, with a reduced occurrence of false positives; the possibility of both retrospective TOF-MS and TOF-MS/MS data analysis to identify previously unknown compounds; no resultant increase in experimental cycle time as the number of compounds of interest increases. The method of acquisition is generic and non-targeted, collecting MS/MS data for all compounds in a given sample throughout the entire LC run, whether known or unknown.

Keywords: Time-of-Flight (TOF) Instrumentation, Rapid Toxicology Screening

P06

Method Validation for a Quantitative Method of 23 Benzodiazepines and 3 'Z-drugs' in 5 Matrices Using DPX WAX Tips and LC/MS/MS

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Introduction: Benzodiazepines and sedatives are some of the most commonly abused drugs in the nation and are often found in cases involving driving under the influence of drugs, sexual assaults and overdoses. Previously at the Orange County Crime Lab, two different extraction methods were used to determine the presence of 22 benzodiazepines and one sedative. Each method required 2mL of blood for a 4 hour extraction that used approximately 20 mL of solvent/sample.

Objective: A new extraction and LC/MS/MS method was developed and validated with higher throughput, lower solvent waste and less sample volume using the SWGTOX Standard Practices for Method Validation in Forensic Toxicology Guidelines.

Method: Initially, 50 µL of deuterated internal standard was added to 0.25 mL of sample (blood, urine, liver homogenate, brain homogenate, or stomach content homogenate) and then protein crashed with 0.75 mL acetonitrile. Samples were then rapidly "cleaned-up" using DPX-WAX tips that selectively remove matrix interferences from the samples by dispersive SPE. The samples were processed by aspirating and mixing the sample solutions with the WAX sorbent using a pneumatic extractor, which allowed up to 48 samples to be processed simultaneously in just a few minutes. The cleaned samples were then diluted with water to match the initial mobile phase on the LC/MS/MS and injected onto a Waters Acquity UPLC coupled to a Waters TQ-S triple quadrupole mass spectrometer utilizing positive electrospray ionization in MRM mode. A Waters BEH C18 1.7 um column (2.1 x 100 mm) held at 40°C with a gradient mobile phase of 0.1% formic acid in water and 0.1% formic acid in acetonitrile at 0.4 mL/min was used for chromatographic separation.

Results: Extraction recoveries for the method were performed for all drugs, except internal standards, resulting in 19 of the drugs having an extraction recovery above 70% and the rest above 60%, across all sample matrices. Limit of detection and quantitation, ion suppression/enhancement, carry-over, and possible interference studies were performed for all of the drugs in the method following the SWGTOX guidelines to streamline the validation. Different regression models were examined to determine the most appropriate model; a quadratic model, weighted $1/x^2$ with no forcing through zero for all drugs, which covered their therapeutic concentrations. The uncertainty of measurement budget for all drugs quantitated were completed during the validation, using over 100 runs and five analysts. For the final validation, four fully trained analysts extracted 50 previously analyzed casework samples, comprised of all matrices, to determine if their results would be within 20% of each other. No deviations greater than 20% were seen.

Conclusion: A quick quantitative extraction and LC/MS/MS method has been developed and validated for 23 benzodiazepines and 3 z-drugs in five different matrices from low therapeutic to fatal concentrations for each drug. The method has been validated using the SWGTOX guidelines and is currently the method being used at the Orange County Crime Lab for antemortem and postmortem casework.

Keywords: Benzodiazepines, Sedatives, Method Development, DPX, LC/MS/MS

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P07

Evaluation of Case History of Synthetic Cannabinoids Detected in Seven Cases in Orange County, CA as Screened by Immunalysis® K2 (Synthetic Cannabinoids-1) Direct ELISA Kit

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Introduction: In March 2011, the United States Drug Enforcement Agency banned five synthetic cannabinoids: JWH-018, JWH-073, JWH-200, CP47,497 (C7), and CP47,497 (C8). Manufacturers and distributers are constantly updating the contents of the herbal blends in order to stay ahead of local and federal legislation. Screening for an ever changing list of synthetic cannabinoids continues to present a challenge to toxicology labs as they try to keep up with what is on the streets. Distributers are constantly updating the contents of the herbal blends in order to stay ahead of local and federal legislation.

Objective: Over 1300 cases, from 2011 and 2012, were screened using Immunalysis® K2 (Synthetic Cannabinoids-1) Direct ELISA Kit to determine if there is a need to include synthetic cannabinoids in routine screening at the Orange County Crime Lab.

Method: The standards used for this study were a 10 ng/mL of JWH-018 5-Pentanoic Acid metabolite cut-off, with a high standard of 200 ng/mL of JWH-018 5-Pentanoic Acid metabolite and a blank of synthetic urine. All samples and standards were diluted 1:10 in phosphate buffer solution (pH 7.0) prior to analysis. After mixing, the samples were analyzed on a Tecan Freedom EVO 150. For the analysis, 10 µL of each sample or standard was pipeted onto an Immunalysis® K2 (Synthetic Cannabinoids-1) ELISA kit. Drug conjugate, 100 µL, was added prior to 60 minute incubation at room temperature, in the dark. The wells were then washed 6 times with 350 µL of water each time and then 100 µL of 3,3',5,5' tetramethylbenzidine and urea peroxide in buffer was added to all wells. The plate was incubated at room temperature for 30 minutes before 100 µL of 1 N hydrochloric acid stop solution was added to each well. The absorbance was measured immediately at 450 nm and 650 nm and the difference between these two readings was calculated. Prior to running casework samples on the kits, a concentration curve was diluted for JWH-018 5-Pentanoic Acid metabolite from 1 – 500 ng/mL in order to ensure that the kit worked at the cut-off concentration desired and that high dose hook effect would not occur at concentrations commonly referred to in literature.

Results: Of the 1314 cases screened, 9 were positive by the ELISA kit and another 2 were within 20% of the cut-off. These cases were then sent to an outside lab for confirmation testing for synthetic cannabinoids. The police or coroner's reports were obtained for all cases and symptomology or cause of death was examined based on all drugs detected, synthetic cannabinoids and others.

Conclusion: From the 1314 cases screened for synthetic cannabinoids by Immunalysis® K2 (Synthetic Cannabinoids-1) Direct ELISA Kit, ~0.5% were positive for at least one synthetic cannabinoid as confirmed by an outside lab. Some of the confirmed synthetic cannabinoids were JWH-210, JWH-122, JWH-018, JWH-022, and AM-2201.

Keywords: Synthetic Cannabinoids, Prevalence Study, DRE, ELISA

P08

Interpretation of Urine Specimens Containing Prescription Drugs without Metabolites: Adulteration, Impaired Metabolism or Normal Excretion?

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Introduction: Noncompliant patients in pain management or treatment may add drug to urine during collection to appear compliant with prescribed therapy. Others may have genetic differences, drug-drug or drug-food interactions, or disease states that impair metabolism. Distinguishing these cases can be problematic for health care specialists.

Objective: To characterize the prevalence of parent-only prescription compounds detected in urine in the absence of metabolites.

Method: The study was approved by an Institutional Review Board. Approximately 38000 urine specimens from chronic pain patients in 35 states were tested by liquid chromatography (LC) tandem mass spectrometry (MS/MS) for 18 opioids, alprazolam, carisoprodol and tramadol and their respective metabolites. A subset of 17730 specimens was tested for buprenorphine/norbuprenorphine, and 829 for zolpidem/metabolite.

Results: The prevalence rates of parent drug in absence of metabolites ≥ reporting threshold are presented in the table. A total of 1124 specimens (2.9%) contained parent-only. Of these, 657 (58.5%) exhibited concentrations over the median concentration for that analyte, with only 116 (10.3%) falling below the 2.5 percentile; 125 (11.1%) specimens yielded detectable concentrations of parent-only for more than one drug. The number of CYP450 metabolic pathways represented by metabolite testing ranged from 1 to 5 (mean 2.1) per specimen. 20 specimens (1.8%) exhibited abnormal results for specimen validity testing including creatinine <2 mg/dL, specific gravity < 1.0020, or pH < 3.5 or >9.

Drug (Reporting Threshold, ng/mL)	# Positives: Parent and/or Unique Metabolite(s)	# Positives Parent-only (%)	# Parent-only Positives Below Median Concentration (%)	# Parent-only Positives Above Median Concentration (%)
Oxycodone (100)	13474	327 (2.4%)	110 (33.6%)	217 (66.4%)
Hydrocodone (100)	11278	178 (1.6%)	106 (59.5%)	72 (40.4%)
Alprazolam (50)	5131	125 (2.4%)	48 (38.4%)	77 (61.6%)
Tramadol (100)	3236	83 (2.6%)	73 (88.0%)	10 (12.0%)
Methadone (200)	2557	126 (4.9%)	44 (34.9%)	82 (65.1%)
Carisoprodol (200))	2531	41 (1.6%)	13 (31.7%)	28 (68.3%)
Fentanyl (5)	2153	138 (6.4%)	57 (41.3%)	81 (58.7%)
Buprenorphine (1)	1881	140 (7.4%)	103 (73.6%)	37 (26.4%)
Codeine (100)	547	34 (6.2%)	30 (88.2%)	4 (11.8%)
Zolpidem (4)	527	69 (13.1%)	22 (31.9%)	47 (68.1%)
Meperidine (100)	84	2 (2.4%)	0 (0%)	2 (100%)
Propoxyphene (200)	49	0 (0%)	0 (0%)	0 (0%)

Conclusion: A significant number of parent-only specimens, many of which contained concentrations above the median, were identified in this study. The absence of detectable metabolite(s) cannot be considered a definitive indicator of specimen adulteration, but raises suspicion of tampering in cases where very high concentrations or multiple parent-only drugs are identified. Specimen integrity must be considered when performing compliance monitoring for these populations; however, drug adulteration may be difficult to detect visually.

Keywords: Pain Management, Metabolism, Interpretation, Urine Tests

P09

Interpreting Unexpected Opiate Results in Oral Fluid: Possible Minor Metabolism or Pharmaceutical Impurity?

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Introduction: Minor metabolism pathways and pharmaceutical impurities may potentially confound interpretation of toxicology results for pain management patients.

Objective: Characterize the relative ratios of minor metabolites and pharmaceutical impurities of opiates in oral fluid specimens for chronic pain patients.

Method: The study was approved by an Institutional Review Board. Approximately 35000 oral fluid specimens from chronic pain patients in 34 states were collected with the Quantisal device and tested by liquid chromatography (LC) tandem mass spectrometry (MS/MS) for codeine (COD), morphine (MOR), norcodeine (NCOD), hydrocodone (HC), hydromorphone (HM), dihydrocodeine (DHC), norhydrocodone (NHC), oxycodone (OC), oxymorphone (OM), and noroxycodone (NOC); limit of quantitation was 1 ng/mL. Specimens were evaluated to determine if prescribed opioids and related metabolites were present in conjunction with a non-prescribed opioid in relatively low concentration, which could potentially be a minor metabolite or pharmaceutical impurity (e.g., HC presence with prescribed OC). The ratio of unexpected minor opioid to the prescribed opioid was tabulated for each potential metabolism and impurity scenario. JMP software was used to plot the frequency distribution of ratios to identify ratios exhibiting increased prevalence. Subgroups of increased ratio frequency, defined as the cluster of ratios reaching between 10 to 100% of maximum frequency, were further characterized.

Results: Five scenarios were explored and are presented in the table below. Ratios mostly fit a log normal distribution but were heavily skewed to the right with outliers, potentially due to patients ingesting the unexpected opioid from a second exogenous source. In most cases, peak ratios approximated previously reported values; however, higher ratios for three pharmaceutical impurities were observed than have been previously suggested.

Unexpected Finding	Potential Source	# Positives Prescribed Drug	# Positives Prescribed Drug with Unexpected Finding	Range of Ratios Subgroup Analysis (N, %)	Ratio, Mean/Median	Highest Frequency of Ratio Distribution
HM	MOR Metabolism	MOR 2217	275	0.0014-0.0483 (235, 10.6%)	0.0156/0.0122	0.0100-0.0150
HC/NHC	COD Metabolism*	COD/NCOD 168	114	0.0030-0.0672 (68, 40.5%)	0.0249/0.0182	0.0100-0.0200
HC/NHC	Pharmaceutical Impurity in OC	OC/NOC 10549	1565	0.0014-0.0547 (622, 5.9%)	0.0162/0.0109 7	0.0025-0.0050
COD/NCOD	Pharmaceutical Impurity in MOR	MOR 3297	305	0.0013-0.0322 (205, 6.2%)	0.0120/0.0099	0.0075-0.0100
OC/NOC	Pharmaceutical Impurity in OM	OM 289	100	0.0027-0.0997 (31, 10.7%)	0.0363/0.0235	0.0116-0.0190

*Additional exclusion criteria: concentrations MOR>COD

Conclusion: A significant number of specimens positive for a prescribed opioid were also positive for an unexpected opioid which could be due to minor metabolism or presence of a pharmaceutical impurity; percentages of affected specimens ranged from 5.9% to 40.5% per prescribed opioid. Pain management practitioners should be advised of these possibilities, and laboratories should be prepared to assist with interpretation when necessary.

Keywords: Opioids, Oral Fluid, Interpretation

P10

Evaluation of an Ultrafast Online SPE/TOF System to Screen for Drugs of Abuse in Forensic Toxicology

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Introduction: Forensic drug screening has traditionally relied on immunoassays and then analytical confirmation with a quantitative method such as GC/MS or LC/MS. The steady increase in the number of samples requiring analysis has created a bottleneck in screening for these drugs across different classes and longer turnaround times for confirmatory tests. While traditional methods are convenient and well established, they are not as sensitive or specific as LC/MS.

Objective: In the present study, we evaluated the ability of an ultrafast SPE/TOF system to screen across different classes of drugs of abuse in urine in a single injection while approaching the sensitivity and accuracy of LC/MS and maintaining the speed and efficiency of a screen (sample cycle times <15 seconds per sample).

Method: Generic mass spectrometry and SPE methods were created for a panel of drugs that span multiple drug classes on a High-throughput RapidFire Mass Spectrometry System interfaced to a 6550 QTOF. Drug-free urine was spiked with a panel of drugs for simultaneous analysis, diluted, and injected for analysis. Samples were loaded onto the SPE cartridge using water with 0.1% formic acid, washed with 5% acetonitrile, and eluted off the cartridge using 50% methanol and 50% isopropanol with 0.1% formic acid. Sample cycle times were under 15 seconds per sample. Data was acquired in MS mode using 2Ghz EDR, high sensitivity, 100-1000mz, and 5 spectra/s. Data analysis was performed using MassHunter Quantitative Analysis B.06.00 software.

Results: A panel of drugs including: gabapentin, pregabalin, tramadol, carisoprodol, methadone, EDDP, PCP, 6-MAM, benzoylecgonine, amphetamines, benzodiazepines (20 analytes) in urine had excellent linearity within their measured ranges with R^2 values greater than 0.995. A single low cutoff value was determined for each analyte (5-100 ng/ml) and combined into a single sample injection. The combined low cutoff sample was injected multiple times and had an accuracy within 15% and a coefficient of variation within 10%. Samples containing different analytes spiked at varying concentrations both above and below the cutoff were tested. This screening method accurately identified positive samples as above the established cutoff concentration. Blinded human samples (100) were analyzed by this SPE/MS/MS methodology and the results were compared to LC/MS/MS values determined for the same samples. Results from the two analytical systems were comparable.

Conclusion: Qualitative analysis of a panel of 20 analytes was quickly, accurately and precisely measured in urine using a simple dilute and shoot method with the potential to add more analytes to this panel. Using this ultrafast SPE/TOF methodology, sensitivity and specificity comparable to LC/MS were achieved without compromising the throughput and speed of traditional methods.

Keywords: Ultrafast SPE/TOF, MassHunter Quantitative Analysis, RapidFire Mass Spectrometry System

P11

Ultrafast, Quantitative Analysis of Buprenorphine, Methadone and Their Metabolites in Human Urine Using Online SPE/MS/MS

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Introduction: Due to the increasing quantity of urine samples analyzed in forensic and research toxicology laboratories; there is a strong demand for higher throughput analysis solutions. An online SPE/MS/MS system enables high throughput analysis of small molecules with sample to sample analysis times of 11-15 seconds. This throughput is >10-fold faster than traditional LC/MS/MS methods.

Objective: The purpose of this study was to develop a confirmation method by online SPE/MS/MS to determine concentrations of drugs of abuse (methadone/EDDP and buprenorphine/norbuprenorphine) in human urine specimens and compare the results to LC/MS/MS.

Method: Buprenorphine/Norbuprenorphine - Calibration standards and quality controls were prepared by spiking drug-free urine with the analytes of interest and deuterated internal standards (buprenorphine-d4 & norbuprenorphine-d3) in a range of concentrations. The samples were then subjected to enzymatic hydrolysis, extracted by a SPE procedure using Agilent Bond Elut 96 Plexa PCX plates, the eluate was dried down and reconstituted in 5 % methanol in water, and then injected onto the SPE/MS/MS system.

Methadone/EDDP - Calibration standards and quality controls were prepared by spiking drug-free urine with the analytes of interest in a range of concentrations. The samples were then diluted with methanol/water (1:1) containing deuterated internal standards (methadone-d3 & EDDP-d3) and injected onto the same SPE/MS/MS system.

Analysis was performed at a rate of <15 seconds per sample using an Agilent RapidFire High-throughput Mass Spectrometry system coupled to an Agilent 6460 triple quadrupole mass spectrometer. SPE methods were optimized for each analyte pair. Data analysis was performed using Agilent MassHunter software monitoring the following MRM transitions: (Bup 468.3→55.1, 396.2m/z), (Bup_d4 472.3 →59.1m/z), (Norbup 414.3→83.1, 101.1m/z), (Norbup_d3 417.3→83.1m/z), (MDN 310.2→265.0, 105.1m/z), (MDN_d3 313.2→268.3m/z), (EDDP 278.2→234.1, 249.1m/z), and (EDDP_d3 281.2→234.1m/z).

Results: This SPE/MS/MS methodology is capable of analyzing more than 240 samples/hour, providing a high-throughput and very efficient mode of analysis for both assays. Buprenorphine/norbuprenorphine and methadone/EDDP standard curves had excellent linearity within the measured range (2.5-400 ng/mL) and (10-5,000 ng/mL) respectively, with an R2 value greater than 0.995. Interday and intraday precision and accuracy values of quality controls (QC) were determined to be within 10% and CV values were all less than 5%. Carryover was assessed, no significant carryover (<10%) was determined for all analytes. Blinded human samples (>100) were analyzed by this SPE/MS/MS methodology and the results were compared to LC/MS/MS values determined for the same samples by a forensic toxicology laboratory. Results from the two analytical systems were comparable. For example, for the 282 samples analyzed for methadone/EDDP, all samples correlated as either positive or negative and for the samples in the linear range the correlation coefficients were >0.975.

Conclusion: Buprenorphine/norbuprenorphine and methadone/EDDP can be measured accurately, precisely and efficiently using a high throughput SPE/MS/MS system. This methodology is capable of throughputs >240 samples per hour with comparable analytical results to LC/MS/MS.

Keywords: Drugs of Abuse, SPE/MS/MS, Methadone, Buprenorphine, High-Throughput, Pain Management

P12
Detection of Alpha-PVP in Postmortem Blood Casework by UPLC/MS/MS

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Introduction: For several years, substituted cathinones have been sold in convenience stores, smoke shops, and on the Internet as "bath salts", "plant food", energy pills and powders, and various "cleaning" products. As these compounds become prevalent and are subsequently controlled by either state or federal governments, newer compounds emerge. In 2012, after the scheduling of methylenedioxypyrovalerone (MDPV) and mephedrone, an uncontrolled compound, α-pyrrolidinopentiophenone, also known as alpha-PVP, emerged. Alpha-PVP is a demethylated derivative of the schedule V federally controlled substance pyrovalerone, a dopamine and norepinephrine transporter inhibitor.

Objective: To develop and validate a rapid ultra-performance liquid chromatography-tandem mass spectrometry (UPLC/MS/MS) method for the quantitative determination of alpha-PVP in human blood specimens.

Method: A 100 µL aliquot of blood specimen underwent a protein precipitation extraction with acetonitrile. Specimens were vortex mixed and centrifuged. A 300 µL aliquot of the organic layer was evaporated to dryness under nitrogen and the residue was reconstituted in deionized water. Ultra performance liquid chromatography was performed via a gradient elution of acidified water and acetonitrile with a C18 stationary phase held at 60°C. Tandem mass spectrometry was performed in positive electrospray ionization mode with multiple reaction monitoring using two transitions and one calculated ion transition ratio for the analyte of interest, resulting in a total analytical run time of 4.5 minutes. A deuterated analog of MDPV was used as the internal standard. The method was validated as a quantitative assay with a linear dynamic range 5-1,000 ng/mL. Accuracy and precision, carryover, exogenous drug interferences, ion suppression, and matrix selectivity were assessed during method validation.

Results: The method has been applied to postmortem toxicology casework received by the forensic laboratory. Postmortem blood specimens of various cause and manners of death resulted in alpha-PVP concentrations ranging from 5-732 ng/mL (mean=317 ng/mL, standard deviation (σ)=227 ng/mL, n=14). In all 14 cases, alpha-PVP was never the sole substance detected. In regards to other toxicology, the most prevalent drug classes included cannabinoids (n=7), opioids (n=6), amphetamines (n=5), and antidepressants (n=4). Other substituted cathinones were detected in 4 of the cases.

Conclusion: Alpha-PVP was an emerging federally uncontrolled novel substance in 2012. The validated UPLC/MS/MS method proved to be accurate and reliable for the detection and quantitation of alpha-PVP in whole blood specimens. The method is applicable to postmortem casework and is routinely used in the forensic toxicology laboratory.

Keywords: Substituted Cathinones, LC/MS/MS, Alpha-PVP, Postmortem Toxicology

P13
Case Report: Identification of 8-Hydroxyquinoline Cannabinoids in Herbal Products

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Introduction: For several years, herbal blends or potpourris have been sold in convenience stores, gas stations and smoke shops. These products have been shown to contain various synthetic cannabinoids. As these compounds are subsequently controlled by either federal or state governments, new compounds are used and become prevalent. In early 2013, we detected a new chemical structure subset of cannabinoid compounds - the quinolinylindoles. Since approximately 2007 and in legitimate academic research, quinoline cannabinoid derivatives have been evaluated for cannabinoid receptor affinity. A few studies have demonstrated a handful of compounds with the quinoline structure to be highly selective for the CB2 receptor over the CB1 receptor.

Objective: To identify quinoline synthetic cannabinoids using the combinatorial approach of ultra-performance liquid chromatography-time of flight mass spectrometry (UPLC/ToF) and ultra-performance liquid chromatography-tandem mass spectrometry (UPLC/MS/MS).

Method: A 5 mL aliquot of acetonitrile:methanol (50:50) was added to 50 mg of plant material specimen. Following a water bath sonication and a vortex mixing, the specimen was diluted with an acetonitrile:DI water mixture, which was injected for analysis.

UPLC/ToF: Ultra performance liquid chromatography was performed via a gradient elution of acidified water and acetonitrile mobile phases on a C18 stationary phase held at 60°C. Time of flight spectrometry was performed in positive electrospray ionization mode over a mass range 30-600 amu. One low voltage scan was performed for precursor mass identification and one higher voltage scan was performed for product mass identification via in-source collision induced dissociation. Total run time was 12 minutes.

UPLC/MS/MS: Ultra performance liquid chromatography was performed via a gradient elution of acidified water and acetonitrile with a C18 stationary phase held at 60°C. Tandem mass spectrometry was performed in positive electrospray ionization mode with multiple reaction monitoring using two transitions and one calculated ion transition ratio for the analytes of interest, resulting in a total analytical run time of 11 minutes. A deuterated analog of JWH-073 was used as the internal standard.

Results: Authentic reference standards were acquired and chromatographic and mass spectral data was determined. The unknown specimens were compared to the authentic reference standards. 5F-PB-22 and PB-22 were detected in ten herbal blend specimens acquired from various locations in Indiana via two complementary mass spectrometry-based methodologies. The compounds were detected as the sole adulterant in the product as well as alongside other synthetic cannabinoids, such as XLR-11.

Conclusion: In early 2013, the 8-hydroxyquinoline cannabinoid derivatives, 5F-PB-22 and PB-22, became emerging compounds in Indiana. At the time of detection, these two compounds were not considered explicitly controlled substances in any state in the United States of America. Currently, they are prevalent in herbal blends and potpourris. Because these have become popularly used, a forensic toxicology laboratory should be monitoring them in synthetic cannabinoid biological assays.

Keywords: Synthetic Cannabinoids, UPLC/ToF, UPLC/MS/MS, PB-22, 5F-PB-22

P14

Development of an ELISA for the Detection of Meprobamate in Human Urine and Blood

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Introduction: Meprobamate (Miltown®, CAS# 57-53-4) was introduced as a drug to treat anxiety, tension, and muscle spasms in humans. Meprobamate is known to metabolize to Hydroxymeprobamate and Meprobamate-N-Glucuronide in humans. Meprobamate abuse is possible and can result in addiction. Meprobamate is currently classified schedule IV by the United States Drug Enforcement Agency (USDEA). Carisoprodol (Soma®, CAS# 78-44-4) is structurally related to Meprobamate and is frequently prescribed as a muscle relaxant. Carisoprodol is extensively metabolized within humans and one of the major metabolites is Meprobamate. Other known metabolites of Carisoprodol include Hydroxycarisoprodol and Carisoprodol N-Glucuronide. Carisoprodol has a potential for abuse and has also recently been reclassified as schedule IV according to the USDEA. At therapeutic levels, both Meprobamate and Carisoprodol can result in drowsiness, fatigue and are frequently encountered in cases involving driving under the influence.

Objective: Develop a highly sensitive enzyme linked immunosorbent assay (ELISA) for the detection of Meprobamate in human urine and blood with useful cross-reactivity with Carisoprodol.

Method: An antiserum was developed against a Meprobamate/Carisoprodol protein conjugate and used to develop a competitive ELISA. The assay was tested for sensitivity, cross-reactivity and matrix interference.

Results: The limit of detection of Meprobamate is 0.01 ng/mL and Carisoprodol is 0.1 ng/mL in assay buffer and <125 ng/mL for Meprobamate and Carisoprodol in diluted human urine and blood. Human urine (n=80) and blood (n=20) sample populations containing no drugs were tested for assay interference and found to be negative at a 250 ng/mL cutoff using a 1:20 sample dilution for human urine and a 1:10 dilution for blood. %Cross-reactivity was tested for Meprobamate, Carisoprodol, Meprobamate-N-ß-D-Glucuronide, and Hydroxymeprobamate. % cross-reactivity was found to be 100, 40.7, 376, and 0.8%, respectively. The assay is highly specific and did not cross-react with a comprehensive selection of other commonly encountered drugs. The reproducibility of the dose response curves were found to be less than ±5%.

Conclusion: The described ELISA is robust and highly sensitive. It can detect Meprobamate at concentrations of 250 ng/mL and higher in both human urine and blood and has sufficient cross-reactivity with Carisoprodol for simultaneous detection in sample matrix The ELISA test offers a rapid and inexpensive screening option suitable for implementation in forensic testing applications.

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Keywords: Meprobamate, Carisoprodol, ELISA, Blood, Urine

P15

A Comparison of Direct Analysis of Morphine in Urine by LC/MS/MS to Traditional Analysis After Acid Hydrolysis

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Introduction: Morphine is a naturally occurring narcotic analgesic used to treat moderate to severe pain. Morphine is predominantly metabolized to morphine-3-glucuronide. After 72 hours about 87% of a dose of morphine is excreted in the urine. Free morphine has been reported to account for approximately 10% of this amount, morphine-3-glucuronide for approximately 75% with lesser amounts of morphine-6-glucuronide and other metabolites.

Method: 123 urine samples submitted from patients receiving morphine were hydrolysed using strong acid and then analyzed on an Agilent 1200 Series Binary Pumps-LX4 Multiplexed HPLC coupled to a Thermo Quantum Access Max MS/MS using a Phenomenex Kinetex C18 column (50 X 3.0 mm, 2.6 µm), with Morphine-D3 as the internal standard (IS). The same specimens were reanalyzed by direct injection (i.e., no hydrolysis) on a Waters Acquity UPLC/Xevo-TQD Mass Spectrometer using a Waters BEH Phenyl column (50 x 2.1mm, 1.7 µm), with Morphine-D6 and Morphine-3-glucuronide-D3 as the internal standards. The analytes measured by the TQD direct injection method were morphine, morphine-3-glucuronide and morphine-6-glucuronide. The concentrations of the glucuronides were reported as morphine equivalents.

Results: All 123 samples were positive for morphine by both methods. The total morphine in the specimens after acid hydrolysis ranged from 104 to 49519 ng/ml. The total morphine in the specimens determined by direct injection, from the sum of the free morphine, the 3 and 6 morphine glucuronide metabolites ranged from 73 to 62465 ng/ml. A concentration comparison of the two methods (Direct Injection results/ Acid Hydrolysis results) ranged from 65 to 158 %, with 56% of the "direct injection" specimens having a greater total morphine concentration. The mean % difference between the two methods was 5% with a standard deviation in the difference of 20.9% demonstrating both the absence of a systemic shift in results and the overall acceptable fit of these data. There was good correlation (R^2=0.957) between the two methods. The relationship of free morphine to the 3-glucuronide and 6-glucuronide in the 74 specimens where all three species were present had the following average distribution: free morphine, 7.36 % ±7.63, morphine-3-glucuronide, 77.57 % ± 8.66, and morphine-6-glucuronide 15.07% ± 7.17.

Conclusion: Total morphine confirmation results for urine specimens by "Direct Injection" gave comparable results to traditional acid hydrolysed values. While the ranges of these data indicate a large difference between the data from each method, the means and standard deviations indicate that most of these data are indeed within 25% of each other for each sample consistent with the validation results for these methods. The preparation of specimens for direct injection was less time consuming and allowed for more information regarding the metabolism patterns of patients. While this presentation discusses findings with patients receiving morphine, the assay allows for the simultaneous determination of morphine, hydromorphone, norhydrocodone, codeine, hydrocodone, oxymorphone, oxycodone, noroxycodone, morphine-3 and 6-glucuronide, hydromorphone-3-glucuronide, codeine-6-glucuronide and oxymorphone-3-glucuronide in 3.9 minutes.

Keywords: Glucuronide, Hydrolysis, LC/MS/MS

P16

Observation of the Rate of Propoxyphene Positive Patient Urine Samples Since Its Withdrawal from the US Market

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Introduction: Propoxyphene (Darvon, Darvocet) is an opioid analgesic that was commonly used in the US to relieve mild to moderate pain. After review of studies showing that propoxyphene was linked with serious abnormal heart rhythms, the FDA recommended that manufacturers remove it from the U.S. market. The FDA also recommended that health care professionals stop prescribing propoxyphene and ask their patients to discontinue using the drug. The FDA announcement was published on November 19, 2010.

Objective: To illustrate the rate of use of propoxyphene in pain patients since November 2010 to allow physicians to determine the medical necessity of continued routine testing for propoxyphene.

Method: Some samples are screened using EIA (Microgenics), but most samples are analyzed at the collection site or physician office using a POCT device. For confirmation analysis, internal standard was added and samples were extracted to prepare them. Analyses were conducted using the Waters ACQUITY TQD UPLC/MS/MS System with the 2.1 × 50mm, 1.7µm Acquity UPLC BEH Phenyl column. A gradient mobile phase system consisting of 2 mM Ammonium Acetate pH 10 and Acetonitrile was used. The method is linear from 50 ng/mL to 20,000 ng/mL, and the reporting cutoff is 100 ng/mL. We examined the test results from all samples analyzed since October 2010.

Results: Ameritox Laboratories performed 223,666 UPLC/MS/MS tests for propoxyphene and nor-propoxyphene on patient urine samples since October 1, 2010.

- The number of propoxyphene tests performed declined from 12,195 in October 2010 to 3,177 in April 2013.
- The number of propoxyphene prescriptions annotated on the requisitions declined from 606 in October 2010 to less than 10 per month in April 2013.
- The number of specimens reported with propoxyphene or nor-propoxyphene positive results has declined from 1,125 in October 2010 to less than 5 per month (0.12%) in February 2013.

Conclusion: We observe that a small number of patients continue to use propoxyphene containing compounds. Many POCT kits still include propoxyphene. Many pain clinics use POCT kits and request confirmation tests to match the analytes included on the POCT kit, regardless of whether the results of the kit indicate a positive result for propoxyphene. The number of UPLC/MS/MS positive samples indicate minimal continued use in the US pain population.

Keywords: Propoxyphene, Nor-Propoxyphene, Pain Management Testing, LC/MS/MS, Point of Collection Test (POCT)

P17

Stereochemical Analysis of R/S -Methamphetamine in Urine Drug Testing by Supercritical Fluid Chromatography

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Introduction: As a separation technique, supercritical fluid chromatography (SFC) has been in existence since the early 1960s. Because of its normal phase chromatographic properties, high pressure operation, and difficulty interfacing to conventional detection techniques, SFC has not experienced overwhelming popularity. However, SFC has found considerable usage in the pharmaceutical field separating chiral compounds with improved sample preparation and a simple 'dilute and shoot' process. Using a Waters ACQUITY UPC^2/MS/MS instrument, the analysis of the R/S isomers of methamphetamine without extraction and derivatisation has been completed. Determination of the clinically relevant ratio of R to S isomer is commonly performed using GC-MS and requires a time- and labor-costly extraction and derivatisation protocol.

Objective: It is our objective to demonstrate the use of ACQUITY UPC^2/MS/MS for the separation and analysis of the R/S forms of methamphetamine using a "dilute-and-shoot" method. Carryover, limit of quantitation (LOQ), Limit of Detection (LOD), and linearity were evaluated.

Method: Five calibration standards were prepared using a racemic mixture of D/L methamphetamine. These points were prepared in urine at the following concentrations: 12, 26, 52, 104, and 156 ng/mL (where concentrations are indicative of each isomer's concentration, *e.g.*, 12ng/mL is 12 ng/mL of R and 12 ng/mL of S). Two methamphetamine mixtures (an 80:20 ng/mL R/S and a 50:50 ng/mL R/S) were run as controls. All standards and eight patient samples were only diluted 1:1 with isopropanol (IPA) prior to injection. Methamphetamine isomers were detected using molecule specific mass transitions (e.g., MRM). A Daicel, Chiralpak AD-H 4.6 x 150 mm 5 µm column was used for separation. Carbon dioxide (CO_2) was used as mobile phase A and IPA with 0.5% isopropylamine was used as solvent B, with a flow rate of 2.5 mL/min over the course of the 9-minute method. A make-up flow was also used to aid in providing a constant solvent flow to the ACQUITY TQD mass spectrometer.

Results: Both isomers show an R^2 value of >0.999 when plotted as peak area vs concentration. The 80/20 R/S control is accurate to within 10% and the 50/50 R/S control is accurate to within 5%. All eight patient samples are correctly observed to contain a predominant amount of R isomer (verified by previous gas chromatograph-mass spectrometry results). The LOD was not determined but is estimated to be <10 ng/mL and perhaps as low as 5 ng/mL. The LOQ for this analysis is 12ng/mL, showing baseline separation and signal-to-noise greater than 10 at this concentration. Carry-over is determined to be negligible in a blank IPA injection following a patient sample injection measured to contain >21,000 ng/mL of R-methamphetamine.

Conclusion: Using the Waters ACQUITY UPC^2/MS/MS system, sample derivatisation and extraction were avoided for the analysis of the R/S isomers of methamphetamine from human urine. No carry-over was detected even following a high sample concentration of >21,000 ng/mL with an LOQ of 12ng/mL. SFC's normal-phase properties afford a 'dilute and shoot' sample preparation for this analysis greatly decreasing time of analysis and lowering costs while maintaining a quality result.

Keywords: Supercritical Fluid Chromatography, R/S-Methamphetamine, MS/MS

P18

Cross-Reactivity of Tramadol Specimens with Tapentadol Enzyme Immunoassay (Immunalysis Corp)

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Introduction: A review of the incidence of positive tapentadol screens for pain management urine specimens using EIA in the absence of a tapentadol prescription, with negative tapentadolconfirmation by UPLC/MS/MS revealed the synthetic narcotic analgesic, tramadol, as the only common drug. Tramadol was not always listed on the associated medication record for the patient samples. The commercial tapentadol immunoassay package insert (Immunalysis Corp, Pomona, CA, USA) indicated evaluation of cross-reactivity with tramadol (0.4% at 50000 ng/mL tramadol), but not for its metabolites. Along with unchanged tramadol, major urinary biomarkers nortramadol and free and conjugated *O*-desmethyltramadol and *O*-desmethylnortramadol are eliminated in urine in comparable amounts.

Objective: A substantial incidence of positive tapentadol screens for pain management urine specimens using a commercial (Immunalysis Corp) enzyme immunoassay (EIA) was observed in the absence of a tapentadol prescription, with negative tapentadol confirmation by ultra-performance liquid chromatography tandem mass spectrometry (UPLC/MS/MS). This work examines the impact of concomitant Tramadol concentrations on the Tapentadol EIA results.

Method: Aliquots of drug-free urine fortified combined or separately with tramadol or one of its major metabolites at various concentrations (100-100,000 ng/mL) were tested against the tapentadolEIA, to evaluate cross-reactivity. UPLC/MS/MS confirmation data for authentic tramadol urine specimens that produced false-positive tapentadol EIA results at a 200 ng/mL cut-off (n=3610) were sequestered and reviewed for tapentadol and metabolites and for tramadol and *O*-desmethyltramadol positivity.

Results: Cross-reactivity was calculated as the percentage of the concentration reading of the tapentadol EIA assay to the lowest concentration of tramadol analyte triggering a positive response. Over five runs, tramadol, *O*-desmethyltramadol and nortramadol exhibited fluctuating cross-reactivity with the tapentadol EIA at 10,000- 2,500 (2.6-8.6%), 25,000-10,000 (1.1-1.7%) and 100,000-50,000 ng/mL (0.3-0.4%), respectively. In the presence of all three analytes combined, cross-reactivity registered between 5000-2500 ng/mL (4.7-17.5%).

Discussion: The variability observed above with tramadol and metabolites was not seen between tapentadol and the tapentadol EIA. All authentic urine specimens confirmed as negative for tapentadol and metabolites, but positive for tramadol and/or*O*-desmethyltramadol. Nortramadol was not monitored in the confirmation method. UPLC/MS/MS detection limits were 50 ng/mL for tramadol and *O*-desmethyltramadol and 100 ng/mL for tapentadol,*N*-desmethyltapentadol, tapentadol sulfate and tapentadol glucuronide. While concentrations of tramadol and *O*-desmethyltramadol ranged from 0 to >100,000 ng/mL, individual concentrations did not always exceed the cross-reactivity limits for each analyte.In a retrospective review of tramadol UPLC/MS/MS positives, an additional set of specimens (n=3094) were found to be positive for tramadol and/or *O*-desmethyltramadol above the highest cross-reactivity limits, but negative for tapentadol EIA. This indicated that an unknown factor could inhibit the tramadol cross-reactivity with the tapentadol immunoassay in some patient specimens.

Conclusion: The cross-reactivity of tramadol and some metabolites with the tapentadol EIA (Immunalysis Corp) was demonstrated using reference standards, with the cross-reactivity for tramadol being higher than previously reported. Despite meeting cross-reactivity limits, a false positive tapentadol EIA screen did not always occur with positive tramadol patient specimens.However, the potential for false-positive results for tapentadol EIA screening of urine specimens containing tramadol, *O*-desmethyltramadol and/or nortramadol must be considered when interpreting results. The incidence is significant enough that it is recommended that all tapentadol EIA positives with negative tapentadol LC/MS/MS confirmations be checked for tramadol.

Keywords: Tramadol, Cross-Reactivity, Tapentadol, Nortramadol, *O*-Desmethyltramadol

P19

Comparison of the Extraction Results of Nine Basic Drugs from Equine Urine Using Agilent TOXI-TUBES and Various Solid Phase Extraction (SPE) Columns

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Introduction: To help ensure drug-free racing in equine graded stakes competitions, extended urine testing protocol is applied. This testing includes additional compounds which cannot always be added to routine screening procedures and additional testing must be performed.

Objective: The extracts from equine urine containing gabapentin, xylazine, levorphanol, clenbuterol, ractopamine, nalorphine, butorphanol, formoterol, and modafinil at concentrations 50, 20, 2, 1, 5, 20, 10, 10, and 20 ng/mL, respectively, using TOXI-TUBES (Agilent Technologies) and six different SPE columns from United Chemical Technologies (UCT), Phenomenex, and Agilent Technologies were evaluated for extraction efficiency and matrix effects.

Method: For Agilent TOXI-TUBES, the basic drug extraction was performed. From UCT, mixed mode silica, polymer, and a strong cation exchange column were tested. From Agilent, a mixed mode polymer and mixed mode weak cation exchange columns were evaluated. From Phenomenex, a polymer bound strong cation exchange column was tested. For each SPE column, the companies' recommended extraction procedures were applied. All extracts were analyzed using LC-MS-MS (Agilent 6400 series LC-MS QQQ). Five mL of urine was used for each extraction following enzymatic hydrolysis in order to provide a better estimate of matrix effects.

Results: Overall, SPE offered greater extraction recovery than TOXI-TUBES for all drugs with a range of 1 to 82%. Agilent's Varian Bond Elut Plexa mixed mode polymer bound column had the most consistent extraction efficiency, ranging from 39% to 65% for all compounds. For the TOXI-TUBES, extraction efficiency for 8 drugs ranged from 8% to 30%, however, gabapentin was unidentifiable in any of the extracts. Matrix effects for TOXI-TUBES ranged from 6% to -90%. Extraction efficiency for modafinil was less than 5% for all columns except for the Agilent mixed mode polymer and cation exchange columns.
Matrix effects for the SPE columns varied from -99% to +140%. However, UCT's silica based strong cation exchange column has the smallest average matrix effect, ranging from -71% to 105%. For SPE and TOXI-TUBES, the signal-to-noise ratios of all targeted ions were greater than 10:1 (except for gabapentin when TOXI-TUBES were used) for all drugs studied.

Conclusion: Although extractions using TOXI-TUBES required less time to perform the extraction as compared to SPE, the results for SPE were superior to TOXI-TUBES. Since gabapentin was unable to be identified using TOXI-TUBES, a separate extraction would have to be employed to detect that drug. Agilent's Varian Bond Elut Plexa column performed the best for the range of drugs tested based on the matrix effects encountered and extraction efficiencies.

Keywords: TOXI-TUBES, SPE, LC-MS-MS, Equine Urine

P20

Analgesia in Birds: Detection of Fentanyl in Chicken Plasma After Transdermal Administration Using LC-MS-MS

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Introduction: Limited information is available regarding analgesia for avian species. Currently, in this taxon, it requires manual restraint or frequent administration. Repeated handling negatively impacts animals which are clinically debilitated. Due to the risks of renal insult, alternatives to NSAIDs have been sought for analgesia. Fentanyl, a potent μ-receptor opioid, is commonly used in domestic mammals but its use parenterally in psittacines revealed an extremely short elimination half-life. However, transdermal fentanyl studies in exotic animals, including rabbits and prehensile-tailed skinks, have revealed that achieved plasma concentrations were comparable to those therapeutic in humans (200 - 2,000 pg/mL).

Objective: The aim of this pilot study was to develop and validate an LC-MS-MS method for quantification of fentanyl and norfentanyl in chicken plasma collected as part of a pharmacokinetic trial for this species as an avian analgesic model.

Method: Four domestic chickens (*Gallus gallus domesticus*) were treated with either a 12.5 mcg/hr (n=2) or 25 mcg/hr (n=2) transdermal fentanyl patch applied to the plucked skin over the muscle dorsal to the ilium. The patch was removed at 72 hours. Blood was collected from the right jugular and ulnar veins at 4, 6, 8, 10, 12, 24, 32, 48 and 72 hours in the lower dose group and at 2, 4, 6, 8, 12, 24, 32, 48, 72, 80, 96, and 105 hours in higher dose group. Plasma was separated within two hours of collection and stored at -80^{o}C until analyzed.
For quantification of fentanyl and norfentanyl in plasma, a liquid extraction method (dichloromethane : ethyl ether : hexanes, 1:1:1, 3 mL) was developed using 250 μL of plasma followed by LC-MS-MS Triple Quad analysis using 3 product ions per compound. Eight point standard curves for fentanyl and norfentanyl were prepared (15-2,000 pg/mL) with deuterated analogs as internal standards. Method validation included stability testing for bench-top, freeze- thaw, processed and long-term storage, interlay and intraday precision and accuracy at three concentration levels (50, 400, and 1,600 pg/mL).

Results: For fentanyl and norfentanyl, break down was 0-19%, and 27-33%, respectively. Interday and intraday precision and accuracy were within $\pm$5% ($\pm$10% at the lower limit of quantitation) of the expected concentrations. Uncertainty was calculated using the Simplified GUM approach, and was 26% for both drugs at 95% confidence level. When the lower dose was administered, fentanyl was detected in plasma throughout the entire study period (concentration range 1,868 - 56 pg/mL). Traces of norfentanyl were detected. In the higher dose group, increased concentrations of fentanyl (6,558 - 30 pg/mL) and measurable concentrations of norfentanyl (255 - 19 pg/mL) were found.

Conclusion: Developed analytical methodology can be employed for quantification of fentanyl and norfentanyl in a wide range of concentrations in chicken plasma. In this pilot study, fentanyl was absorbed from the patch through the avian skin reaching plasma concentrations that have pharmacological action in other species.

Keywords: Fentanyl, Norfentanyl, Chicken, Plasma, LC-MS-MS

P21

Multi Class Drugs of Abuse Extraction from Urine Using Supported Liquid Extraction Prior to UPLC/MS-MS Analysis

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Introduction: Drugs of abuse screening 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. For example, strong cation exchange SPE can extract benzodiazepines, however, as an ionic interaction is not present, the rigorous washing protocols cannot be used. As a result extract cleanliness is compromised. Supported liquid extraction allows for the simultaneous analysis of cross functional analytes in a single extraction protocol without forfeiting extract cleanliness.

Objective: This poster aims to describe simplified sample preparation procedures for multi class drugs of abuse screening prior to LC/MS analysis.

Method: A panel of 44 drugs of abuse and selected metabolites comprising benzodiazepines, Z-drugs, amphetamines, cathinones, opiates, cocaine and BZE, buprenorphine, fentanyl and ketamine was spiked into urine at concentrations from 1-500 ng/mL. Extraction was performed from hydrolyzed and unhydrolyzed urine. Hydrolysis was performed using an established enzymatic protocol involving ß-glucuronidase enzyme at pH5. It was necessary to raise the pH of the sample with concentrated NH_4OH post hydrolysis, prior to SLE extraction. Unhydrolyzed urine was modified using 1% NH_4OH for effective pH control. Extraction was investigated using a range of commonly used water immiscible organic solvents: MTBE, DCM, 95/5 DCM/IPA and EtOAc. Initial method development was performed extracting 100 µL of urine using a 96-well plate format before demonstrating method scalability allowing for the extraction of larger matrix volumes on 1 mL columns. Calibration was performed using a number of isotopically labelled internal standards. All extracts were analysed using a Waters ACQUITY UPLC system coupled to a Quattro PREMIER XE triple quadrupole mass spectrometer. Chromatography was performed using a Waters BEH C_{18} UPLC column. As this method is designed as a screening approach a single MRM transition was acquired for each analyte using electrospray ionisation in the positive ion mode.

Results: Optimal extraction was observed using slightly basic pH modification for both hydrolyzed and unhydrolyzed urine in combination with an extraction solvent of 95/5 DCM/IPA. Recoveries greater than 90% and corresponding RSDs below 10% were typical. The extraction procedure was scaled up from the 96-well plate format to 1 mL columns in order to increase matrix volume extraction. Excellent scalability in terms of recoveries and RSDs were observed with almost all analytes showing < 5% deviation between formats. Calibration curves were constructed from 1-500 ng/mL for both formats extracting 100 µL of both hydrolyzed and unhydrolyzed urine on the 96-well plate compared to 500 µL on 1 mL columns. Good linearity was achieved for most analytes demonstrating coefficients of determination > 0.99. However, quadratic function was observed for a number of analytes at high concentrations. This was corrected using subsequent dilution. Sub ng/mL levels were achieved for extractions from 100 µL of urine using the 96-well plate format.

Conclusion: This poster provides a simple and reliable protocol for the simultaneous extraction of multiclass drugs of abuse from hydrolyzed and unhydrolyzed urine, using supported liquid extraction prior to UPLC/MS-MS. The methodology demonstrated high reproducible extraction efficiencies, good coefficients of determination and acceptable limits of quantitation.

Keywords: LC-MS/MS, Drugs of Abuse, Urine Screening

P22

Extraction of Cocaine & Metabolites from Urine and Oral Fluid Using Supported Liquid Extraction (SLE) Prior to GC/MS and LC/MS-MS Analysis

Lee Williams*[1], Rhys Jones[1], Adam Senior[1], Geoff Davies[1], Helen Lodder[1], Steve Jordan[1], Gavin Jones[1], Claire Desbrow[1], Victor Vandell[2], Frank Kero[2], and Elena Gairloch[2]; [1]Biotage GB Limited, Cardiff, UK, [2]Biotage, Charlotte, NC, USA

Introduction: Cocaine is one of the most widely abused illicit drugs and its abuse is not confined to any particular socio-economic class. This widespread misuse has led to the necessity of rapid and reliable methods for analysis and quantitation from various matrices.

Objective: To develop an assay for the determination of cocaine and metabolites from urine using supported liquid extraction (SLE) applicable to both GC/MS and UPLC/MS-MS analysis. The SLE extraction mechanism is very efficient, delivering higher analyte recoveries and cleaner extracts than equivalent LLE methods.

Method: Blank urine was spiked with a panel consisting of cocaine, norcocaine, BZE, BZE-d_3, EME, AEME and cocaethylene. Initial method development was performed by extracting 100 µL of unhydrolyzed urine using the 200 µL SLE+ 96 fixed well plate format, before demonstrating method scalability on the 1 mL columns, which allows for the extraction of larger matrix volumes. Various buffers were evaluated for effective pH control by 1:1 (v/v) pre-treatment with the spiked urine. A number of commonly used water-immiscible organic solvents were used to evaluate cocaine and metabolite extraction efficiencies: MTBE, DCM, 95/5 (v/v) DCM/IPA and EtOAc. Latter stages of method development involved hydrolyzing urine samples using an established enzymatic method.

Extracts were analysed using a Waters ACQUITY UPLC system coupled to a Quattro PREMIER XE triple quadrupole mass spectrometer. A single quantifier and qualifier MRM transition for each analyte was acquired using positive electrospray ionisation. Method transferral was investigated to incorporate the potential for GC/MS analysis. Again, hydrolyzed and unhydrolyzed urine was investigated, along with oral fluid matrix sampled with the Quantisal collection device. Blank oral fluid specimens mixed with Quantisal buffer were spiked with cocaine, cocaine metabolites and BZE-d_3. The pH environments of the Quantisal buffer/oral fluid and the hydrolyzed urine were controlled with concentrated ammonium hydroxide, prior to SLE extraction. Extraction conditions for the GC/MS end point were evaluated using the 1 mL format as in the LC/MS-MS development and also a smaller 400 µL format. Full extraction and derivatisation details will be shown in the final poster. All samples were analyzed using an Agilent 7890 GC coupled to a 5975 MSD.

Results: For LC/MS-MS, recoveries greater than 90% were observed for cocaine, norcocaine and cocaethylene using pH pre-treatments with 100 mM NH_4OAc (no pH control), 0.1%, 1% and 2% NH_4OH with all extraction solvents. Benzoylecgonine required DCM as the extraction solvent for any recovery, but optimal performance demonstrating recoveries greater than 70% was observed using the more polar 95/5 DCM/IPA combination, following a 0.1% NH_4OH pre-treatment. Linearity was acceptable; results demonstrating coefficients of determination greater than 0.997 over the concentration range 1-100 ng/mL. For GC/MS, recoveries greater than 80% were observed for all analytes across hydrolyzed, unhydrolyzed and oral fluid matrices. Linearity was measured to be greater than 0.995 over concentration range 25-200 ng/mL.

Conclusion: This poster provides a simple and reliable protocol for the extraction of cocaine and metabolites from urine and oral fluid, in both 400 µL and 1 mL load applications. With both GC/MS and UPLC/MS-MS approaches, high, reproducible extraction efficiencies and acceptable limits of quantitation are also demonstrated.

Keywords: GC/MS, LC-MS/MS, Cocaine and Metabolites, Urine, Oral Fluid

P23

Analysis of Antiepiletic Drugs in Serum by LC/MS/MS Using Biotage Isolute® SLE+ (Supported Liquid Extraction)

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Introduction: The purpose of this procedure is to quantitate antiepileptic drugs (AED's) for Therapeutic Drug Monitoring (TDM). TDM of AED's is used to establish toxicity and efficacy of treatment since clinical response correlates better to concentration of drug rather than dosage.

Objective: Validate a method to quantitatively measure five AED's in serum using BIOTAGE Isolute® SLE+ (Supported Liquid Extraction) followed by LC/MS/MS analysis.

Method: Sample preparation involved a "salting out" technique using saturated sodium chloride and saturated ammonium sulfate. The samples were then loaded onto the Biotage Isolute® SLE+ plate and AED's were eluted with Ethyl Acetate. The eluent was then dried and reconstituted with 20/80/0.1% (Methanol/HPLC H_2O/Formic Acid). A Shimadzu Prominence LC coupled with an ABSciex 3200® MS/MS was utilized to perform the analysis. The LC separation was performed with a Restek Ultra Biphenyl 5µm 50 x 2.1 mm column. Mobile phases consisted of: A (0.1% formic acid in HPLC water) and B (0.1% formic acid in methanol) and was run with a 8.0 minute gradient of 10% B to 98% B at a flow rate of 0.8 mL/min. MS detection and quantitation were acquired by Multiple Reaction Monitoring (MRM) in positive ion mode using electrospray ionization. Each run included a five-point calibration curve and two external controls obtained from UTAK Laboratories, Inc.

Results: The patient results were compared to a reference laboratory and yielded mean percent accuracies of: Carbamazepine (CBZ) 98% (n=12), Lamotrigine (LAMO) 106% (n=18), Levetiracetam (LEVT) 98% (n=18), Topiramate (TOPR) 95% (n=10), and Monohydroxycarbamazepine (MHC) 103% (n=15). Between run precisions (%RSD) at the limit of quantitation (LOQ) were: CBZ 5.6%, LAMO 6.8%, LEVT 5.7%, TOPR 4.6%, and MHC 5.5%. Correlation coefficients ranged from 0.9993 to 0.9999 for all analytes, which produced an analytical range of 0.5 mcg/mL to 50mcg/mL for CBZ, LAMO, and TOPR and 1.0 to 100 mcg/mL for LEVT and MHC. Ion suppression studies were performed and showed no interference with the analytes of interest in serum/plasma or whole blood.

Conclusion: A protocol for the quantitation of five AED's in serum was developed using Biotage Isolute® SLE + and LC/MS/MS. The method is simple, rapid, and can provide clinicians with patient results for multiple AED's in one assay.

Keywords: Antiepileptic Drugs, LC/MS/MS, SLE+ (Supported Liquid Extraction)

P24

Extraction of Substituted-Cathinones (Bathsalts) from Urine Using Supported Liquid Extraction (SLE) Prior to GC/MS Analysis

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Introduction: Designer substituted-cathinones or bathsalts, have come to stand out in recent years as drug users look to this legal-grey area for new drugs. Lawmakers in many countries have outlawed mephedrone, methylone and methylenedioxypyrovalerone (MDPV), three substances which are commonly used to make so called "bathsalts" and "plant food", but "street" chemists are fast to design similar chemicals with the aim of mimicking the effects of the banned substances. This growing misuse has led to the necessity of rapid and reliable methods for analysis and quantitation.

Objective: The objective was to develop a GC/MS assay for the determination of substituted cathinones from urine using supported liquid extraction (SLE). The SLE extraction mechanism is very efficient, delivering higher analyte recoveries and cleaner extracts than equivalent LLE methods.

Method: Blank human urine was spiked with a panel of methcathinone, mephedrone, methedrone, methylone, butylone, ethylone, MDPV and naphyrone. In addition, ethylone-d_5 was used as the internal standard. Extraction conditions were evaluated using spiked urine pre-treated 1:1 (v/v) with 1% ammonium hydroxide (150 mM). Sample preparation was performed on a 1 mL SLE+ column using 1 mL of pre-treated urine. Extraction was evaluated using ethyl acetate or MTBE as the solvent of choice. 100 µL of 200 mM HCl was added to each sample after extraction to evaluate the stability of the more volatile compounds during blowdown. Samples were blown down with air below 25°C and derivatization was performed using 50 µL pentafluoropropionic acid anhydride (PFPA) and 50 µL ethyl acetate. The samples were vortex mixed and transferred to glass vials with non-split caps and heated to 70°C for 20 minutes. The samples were then cooled and blown down with air below 25°C and reconstituted with 100 µL 95/5 (v/v) DCM/IPA prior to GC/MS analysis. All samples were analyzed using an Agilent 7890 GC with a 5975 MSD. Chromatography was performed on a BPX-5 SGE capillary column; 30 m x 0.25 mm ID x 0.25 µm using 1.2 mL/min helium as the carrier. Positive ions were acquired using electron ionization operated in SIM mode.

Results: The presence of HCl proved to be essential for methcathinone, mephedrone, methedrone and methylone in blowdown comparison experiments. Butylone, ethylone, MDPV and naphyrone were not adversely affected when HCl was absent.

Limits of quantitation were measured to be the following: 5 ng/mL for methcathinone, methedrone, methylone and MDPV and 10 ng/mL for mephedrone, butylone, ethylone, and naphyrone. In order to increase the sensitivity, the reconstitution solvent volume could be evaluated. Peak areas of the analytes from spiking before and spiking after extraction were measured to determine a percentage matrix recovery profile using SLE+. Recovery profiles were determined to be greater than 80% for all analytes in 3 urine donors and RSDs below 10%. Linearity was acceptable demonstrating coefficients of determination greater than 0.99 over concentration levels 10-500 ng/mL.

Conclusion: This poster provides a quick, simple and reliable protocol for the extraction of substituted cathinones from urine prior to GC/MS, demonstrating high, reproducible extraction efficiencies and acceptable limits of quantitation from multiple urine donors. This fulfils what was set in the objective stage.

Keywords: Supported Liquid Extraction, Bath Salts, Urine, GC/MS

P25

Extraction of Opiates from Urine Using Supported Liquid Extraction (SLE) Prior to GC/MS Analysis

Rhys Jones*[1], Lee Williams[1], Adam Senior[1], Geoff Davies[1], Helen Lodder[1], Steve Jordan[1], Gavin Jones[1], Claire Desbrow[1], Victor Vandell[2], Frank Kero[2], and Elena Gairloch[2]; [1]Biotage GB Limited, Cardiff, UK, [2]Biotage Charlotte, NC, USA

Introduction: Opiates are well known compounds in toxicology that continue to be abused for their sedative and pain relieving effects. Given that there are both genuine and illicit reasons for the presence of opiates in the biological system, a sensitive, specific assay is required to determine individual compounds and thus specific circumstances between cases. When opiates are metabolized in the body, substances such as morphine exhibit significant glucuronidation. As a result a hydrolysis pre-treatment stage is required to cleave the glycosidic bond and allow for complete quantitation in the urine sample.

Objective: The objective was to develop a GC/MS assay for the determination of opiates from urine using Biotage ISOLUTE SLE+ (supported liquid extraction). The SLE extraction mechanism is very efficient, delivering higher analyte recoveries and cleaner extracts than equivalent LLE methods. Additionally the objective was to reach levels below SAMHSA guidelines in urine of 2000 ng/mL for parent opiate compounds and 10 ng/mL for the metabolite 6-MAM.

Method: Blank urine was spiked with an opiate panel consisting of dihydrocodeine, oxycodone, oxymorphone, codeine, morphine, hydrocodone, hydromorphone and 6-MAM. In addition, morphine-d_3 and 6-MAM-d_3 internal standards were spiked into the urine prior to hydrolysis.

Extraction conditions were evaluated using spiked urine, pre-treated (1:1; v/v) with 100 mM ammonium acetate pH 5. Samples were hydrolyzed using 50 µL (approx. 4500U) ß-glucuronidase for 2 hours in a water bath at 60°C. Post-hydrolysis, samples were adjusted using 10 µL of 25% NH4OH (3.75M) resulting in an approximate urine pH of 8. Sample preparation was performed on a 1 mL SLE+ column using 1 mL of pre-treated urine. Extraction was performed using 2 x 2.5 mL volumes of 95:5 (v/v) DCM:IPA. Samples were blown down with air and derivatized with 20 µL BSTFA:TMCS 99:1 and 20 µL 95:5 DCM:IPA. The samples were transferred to glass vials with non-split caps and heated to 70°C for 30 minutes prior to GC/MS analysis.

All samples were analyzed using an Agilent 7890 GC with a 5975 MSD. Chromatography was performed on a BPX-5 SGE capillary column; 30 m x 0.25 mm ID x 0.25 µm using 1.2 mL/min helium as the carrier. Positive ions were acquired using electron ionization operated in SIM mode.

Results: Analyte peak areas from samples that were spiked before and after extraction were compared to determine percentage recovery when using the SLE procedure. A range of 2 ng/mL to 1000 ng/mL was investigated. Recovery profiles were determined to be greater than 70% for all analytes in 3 urine donors and RSDs below 10%. Linearity was acceptable demonstrating coefficients of determination greater than 0.99 over concentration levels 2-1000 ng/mL. Limits of quantitation were: 6-MAM 2 ng/mL, codeine 40 ng/mL, dihydrocodeine 40 ng/mL , morphine 100 ng/mL, oxycodone, oxymorphone, hydrocodone and hydromorphone were all 200 ng/mL. In order to increase the sensitivity, the reconstitution solvent volume could be evaluated.

Conclusion: This poster provides a simple and reliable protocol for the extraction of opiates from hydrolyzed urine prior to GC/MS, demonstrating high, reproducible extraction efficiencies from multiple urine donors. In addition, the extracted concentrations reached with this method are below the SAMHSA guidelines; therefore this fulfils what was set in the objective stage.

Keywords: Supported Liquid Extraction, Opiates, Urine, GC/MS

P26

A Fast Sample Preparation Strategy Towards the Extraction of Illicit Drugs from Urine and Hemolyzed Whole Blood Using Supported Liquid Extraction Prior to HPLC-MS/MS Analysis

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Introduction: The abuse and illegal misuse of schedule I and pharmaceutical drugs is an ongoing problem that sometimes results in the death of the user.

Objective: A fast and reliable testing protocol is needed to address complex matrices typically encountered for post-mortem testing. A fast, reliable sample preparation method which could be implemented to extract a broad range of drugs from completely hemolyzed whole blood and aged urine samples would aid post-mortem toxicology labs in expediting drug toxicology testing on deceased subjects. Here, we demonstrate a new rapid and reliable sample preparation method called supported liquid extraction (SLE) to extract a broad suite of drugs from extremely complex matrices. Qualitative and quantitative data demonstrates utility of this method prior to LC-MS/MS analysis.

Method: SLE in a 96 fixed well plate format was used to extract blank human urine (hydrolyzed and un-hydrolyzed) and hemolyzed whole blood spiked with a multi-drug standard mixture at concentrations from 10.0-0.25 ng/mL. Sample pre-treatment consisted of a 1:1 dilution of urine (200µL) with 2% ammonium hydroxide (200µL). An optimal extraction solvent of ethyl acetate was identified. Extracts were evaporated to dryness, reconstituted in mobile phase and injected onto an Agilent 1200 coupled to a Applied Biosystems 4000 Q-trap triple quadrupole mass spectrometer. A Biotage Resolux C_4 column (4.5µ, 150 x 2.1 mm i.d.) held at 60 °C with a gradient mobile phase of 0.1% ammonium hydroxide/ 0.01% formic acid in water and 0.01% formic acid in acetonitrile at 0.5 mL/min was used for chromatographic separation. Positive electrospray ionization in MRM mode was utilized.

Results: Blank urine was spiked, hydrolyzed with ®-glucoronidase, pre-treated and extracted on the SLE plate. The samples were extracted at concentrations ranging from 2.5 ng/mL-0.25 ng/mL. Averaged recoveries of greater than 85% with intra-run %RSDs less than 10% was observed for all of the target analytes. The addition of 50mM HCL (50µL) to each collection well prior to sample elution aided in decreasing sample degradation (specifically methamphetamine) during the dry down process. Hemolyzed whole blood, which was used to simulate post-mortem blood, was spiked with the multi-drug suite at a concentration of 10ng/mL. The target analytes were recovered at efficiencies ranging from 60%-104%. Calibration curves were generated for the analytes in urine and used to calculate the concentrations of QC standards to within ±15% of the known values.

Conclusion: We present a fast simplified approach for extraction of multiple drugs with reproducible recoveries for low analyte detection levels using a minimal amount of urine (200 µL) or blood (500µL).

Keywords: Supported Liquid Extraction, Multi-Drug Screening, Sample Preparation, Whole Blood

P27

A Novel Extraction Method for the Extraction of Illicit Drugs from Oral Fluid Using Supported Liquid Extraction Prior to HPLC-MS/MS Analysis

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Introduction: Drug of abuse testing is typically conducted using urine specimens collected from patients being screened for illicit drug use.

Objective: While collection of urine is considered a relatively non-invasive drug testing action, it is not conducive for implementation by law enforcement in the field. The ability to collect an oral fluid sample from a person for drug screening in the field can be considered non-invasive and simple to implement. Currently there are oral fluid collection kits (e.g. Immunalysis Quantisal™, Orusure Technologies Intercept®) that facilitate collection of oral fluid specimens. These kits preserve the oral fluid in a buffer solution. Oral fluid samples collected using standard collection kits can be qualitatively and quantitatively analyzed by LC-MSMS post extraction of the target analytes from the matrix. A fast and reliable sample preparation method using supported liquid extraction (SLE) was developed to extract a mixture of basic drugs (e.g. opiates and benzodiazapines) and/or synthetic cannabinoids (Spice) from spiked neat oral fluid and patient oral fluid samples collected with the collection kit devices mentioned above.

Method: SLE in a 400uL load cartridge format was used to extract synthetic cannabinoid parent and metabolites and basic drugs from a minimal amount of oral fluid collected as a neat solution or collected using an oral fluid collection devices. The analytes were spiked into the oral fluid (neat and buffered) at concentrations ranging from 10-50ng/mL. The samples were pre-treated via a 1:1 dilution of spiked neat oral fluid or oral fluid in buffer solution (200µL) with water (200µL) or 2% ammonium hydroxide (200µL) for Spice and basic drugs, respectively. All of the target analytes were extracted with less than 2mL of ethyl acetate. Extracts were evaporated to dryness, reconstituted in mobile phase and analysed via a Biosystems 4000 Q-trap triple quadrupole mass spectrometer with an Agilent 1200 liquid chromatographic system. Positive electrospray ionization in MRM mode was utilized to analyze samples.

Results: The ease of use for handling and extracting samples using the oral collection kits was noted. A minimal volume of 200µL of oral fluid was successfully extracted using SLE. The analytes that were spiked into oral fluid as a neat solution or mixed with buffer from the oral collection devices and were recovered at efficiencies ranging from 65% - 110%. The intra-run %RSDs were less than 10%.

Conclusion: We present a fast simplified approach for extraction of basic drugs and Spice drugs with reproducible recoveries at low analyte detection levels using a minimal amount of oral fluid (200 µL).

Keywords: Supported Liquid Extraction, Multi-Drug Screening, Sample Preparation, Oral Fluid

P28

Confident Identification of Drugs in Post-Mortem Urine and Serum Using High Resolution Mass Spectrometry

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Introduction: High resolution mass spectrometry became an excellent tool in forensic toxicology because of the capability to screen for hundreds or thousands drugs that may cause poisoning at the same time and the capability to identify true unknown compounds using accurate mass MS/MS data.

Objective: Known retention times and ionization behavior enable efficient and reliable detection with significantly reduced numbers of false positive results compared to using databases without such knowledge. For further reduction of false positives the concept of using “diagnostic ions”, e.g. adducts, isotope or fragment ions, has proven to be useful [1]. Therefore in this work various options for enhanced result confirmation like diagnostic ions or fragments generated in broad-band CID are tested to achieve maximum result affirmation.

Method: A set of 61 compounds of forensic interest were selected on basis of practical relevance in post-mortem and routine drug screening, covering a variety of compound classes plus the full range of relevant properties (exact mass, retention time, fragmentation energy). Solvent based mixes were run on identical systems at all three sites (Dionex RSLC with Acclaim C18 column, coupled to a Bruker Daltonics impact ESI-QTOF) using given methods and data acquisition in alternating full-scan/bbCID mode to build up a screening database containing name, sum formulae and RT, plus fragments observed in bbCID mode. Urine and serum samples (after ACN precipitation) were spiked with these compounds at levels ranging from 10 – 500 ng/ml and analyzed on the system in Bremen. The total numbers of findings in all samples were collected, thus counting also the events of false positives. The same processing was applied to data for authentic samples from routine screening cases which were run on the systems in Helsinki (11 urine samples; autopsy cases) and Freiburg (7 urine or serum samples; post mortem & roadside testing cases). Results were compared to findings from established routine screening methods.

Results: The method works well for the selected set of forensic compounds. The compounds are evenly spread across the chromatogram, with good peak shapes also for early eluting compounds (ecgonine methyl ester, morphine) and compounds known for chromatographic issues (bromazepam, olanzapine). RT values were stable throughout the complete sequence and independent from matrix. RT reproducibility between all three sites was <0.2min (0.35 min for olanzapine). Most of the spiked compounds can be completely identified, only pregabalin and carboxy-THC are missed at 10 ng/ml level. Due to the low intensity thresholds, a significant number of false positives are observed even in blank samples (up to 24 in urine blank). However, after applying the diagnostic ion criteria for detection, about two third of the random findings are removed by the criterion of detecting an isotope. After additionally taking into account the bbCID data, only caffeine (urine and serum) and paracetamol (urine) are left as plausible true positive findings (detailed statistic to be given in the presentation).

Conclusion: The results for the authentic case samples were in perfect agreement with findings from routine analysis with no false positive findings.

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Keywords: High Resolution Mass Spectrometry, Time-of-Flight, LC-MS/MS, Drug Screening

P29

In Vitro Inhibition of Buprenorphine Cytochrome P450-Dependent Metabolism by H_2-Receptor Agonists: Cimetidine is a Time-Dependent Inhibitor

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Introduction: Buprenorphine is an opioid used to treat opioid dependence and with increasing frequency for pain management. Buprenorphine is a partial mu-receptor agonist that displays a ceiling effect in regards to many opioid-like clinical effects. For this reason it is considered a safer opioid and in the US is a Schedule III while many other opioids are Schedule II. Buprenorphine, however, is associated with drug-related fatalities, albeit far fewer than seen with methadone and oxycodone. Buprenorphine is N-dealkylated to norbuprenrophine by cytochrome P450s (CYPs) 3A4 and 2C8. As such, it is subject to potential drug interactions arising from CYP inhibition. We have now included buprenorphine in our current studies on prediction of drug interactions from in vitro metabolism.

Objective: To determine the effect of H_2-receptor antagonists on the in vitro metabolism of buprenorphine.

Method: Incubations were conducted with human liver microsomes (HLMs) or cDNA-expressed CYPs (rCYPs). Incubations were conducted either with or without preincubation of inhibitor. For 'without', buffer, enzyme source, buprenorphine and inhibitor were mixed and the reaction was initiated by addition of an NADPH generating system (NADPH-GS). For 'with', buffer, enzyme source, and inhibitor were mixed and reaction initiated by addition of an NADPH-GS with buprenorphine added 15 minutes later. For detailed time-dependent inhibition kinetics, a primary and secondary incubation system were used. CYPs were incubated with just inhibitor and NADPH-GS; at specified times, aliquots were removed and added to a secondary system that had additional buffer, buprenorphine and NADPH-GS. Inhibitor and enzyme were 10-fold diluted. The secondary system was incubated for a set amount of time. Incubation reactions were terminated by addition of methanol. Norbuprenorphine formation was determined using our liquid-liquid extraction liquid chromatography tandem mass spectrometry method.

Results: The H_2-receptor antagonists cimetidine, famotidine, nizatidine and ranitidine were first incubated with HLM at 0, 10, 300 or 1000 μM under with or without preincubation conditions. Both cimetidine and famotidine produced concentration-dependent inhibition that approached or exceeded 50% of control at the higher concentration. The inhibition by cimetidine was greater after preincubation. Neither nizatidine nor ranitidine produced noticeable inhibition of norbuprenorphine formation. IC_{50}s were then determined for cimetidine and famotidine with CYP3A4 and 2C8 were in the 450-1200 μM range, which does not appear clinically relevant. Preincubation of cimetidine was associated with a leftward (more potent) shift in IC_{50}s for both CYP3A4 (450 to 180 μM) and CYP2C8 (930 to 500 μM). Time-dependent inhibition kinetic studies revealed preincubation time- and inhibitor concentration-dependent inhibition of norbuprenorphine formation by both CYP3A4 and 2C8. For CYP3A4, the K_i was 31.0 μM and k_{inact} 0.668 min^{-1}; for CYP2C8, the K_i was 76.8 μM and k_{inact} 0.00114 min^{-1}.

Conclusion: Cimetidine and famotidine produce reversible inhibition of buprenorphine N-dealkylation to norbuprenorphine. The high IC_{50}s reported here and elsewhere were not consistent with known in vivo inhibitions by cimetidine. The in vivo effect was partially explained by the demonstration of time-dependent irreversible inhibition of CYP2D6 by cimetidine (Madiera et al., 2004). We have now shown that cimetidine also produces time-dependent inhibition of CYP3A4 and 2C8.

References:

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Keywords: Buprenorphine, Drug Interaction, Time-Dependent Inhibition, Cimetidine, H_2-Receptor Antagonists

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P30

In Vitro Inhibition of Methadone and Oxycodone Cytochrome P450-Dependent Metabolism: Reversible Inhibition by H_2-Receptor Agonists and Proton Pump Inhibitors

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Introduction: There has been an alarming increase in mortalities associated with opioid use and abuse. Forensic toxicology findings have indicated that drug interactions may contribute to the mortality. Oxycodone and methadone are two such opioids. Both are metabolized by cytochrome P450s (CYPs) that would be subject to drug interactions.

Objective: We propose that in vitro inhibition studies can predict potential pharmacokinetic drug interactions.

Method: Inhibitors, at 3 concentrations, were first incubated with human liver microsomes (HLM), a source of NADPH and methadone or oxycodone. Incubations were conducted with or without preincubation of inhibitor with the enzyme and NADPH to test for time-dependent inhibition. Those that produced ≥ 50% inhibition were then incubated with the respective cDNA-expressed human cytochrome P450s (rCYPs) and 6 concentrations of inhibitor to determine the IC_{50}. R- and S-EDDP formation and noroxycodone and oxymorphone formation were determined by separate liquid chromatographic-tandem mass spectrometric methods. The current studies considered four H_2-receptor antagonists and five proton-pump inhibitors (PPIs).

Results: The H_2-receptor antagonists, cimetidine and famotidine (10-1000 μM), inhibited all four pathways >50%. Nizatidine and ranitidine did not. All five PPIs (1-200 μM) inhibited one or more pathways >50%. IC_{50}s were then determined using rCYPs and K_is then estimated using the Cheng-Prusoff equation (1):

(1) $K_i = IC_{50}/(1 + S/K_m)$

In vivo potency, AUC_i /AUC_n, (the ratio of area under the concentration curve in presence of inhibitor (i) versus no inhibitor (n)), was estimated using a standard equation that requires inhibitor plasma concentration [I] (2):

(2) $AUC_i /AUC_n = 1 + [I]/K_i$,

We considered both therapeutic [I] taken from the literature and 10X[I] to mimic toxic conditions. An AUC ratio ≥ 2 is generally considered significant. Cimetidine and famotidine did not exceed 2 with any of the pathways, even at 10X therapeutic concentrations. For several of the PPIs the ratio exceeded 2 at therapeutic concentrations. Notable inhibitions were (ratio in parentheses): oxycodone metabolism by CYP3A4 and pantoprazole (13), omeprazole (3.3) and esomeprazole (3.0); oxycodone metabolism by CYP2D6 and lansoprazole (5.4); R- and S-methadone metabolism by CYP3A4 and omeprazole (3.5, 3.6), esomeprazole (2.0, 2.1) and pantoprazole (2.7, 3.2). Methadone metabolism by CYP2B6 did not exceed 2 with either therapeutic or 10X concentrations.

Another consideration in scaling in vitro inhibition is the extent of protein binding of the substrate and inhibitor such that only free (unbound) concentrations in plasma ($f_{u,pl}$) (from literature) and in enzyme source ($f_{u,HLM}$ or $f_{u,rCYP}$) (hypothetical) are used. We modeled a few variations, in brief, When $f_{u,pl}$ is small and $f_{u,rCYP}$ is negligible, the ratio decreases dramatically. When $f_{u,pl} \approx f_{u,rCYP}$ the ratio is essentially the same as when binding was not considered.

Conclusion: Several PPIs demonstrated reversible inhibition of one or more of the pathways that may prove significant under in vivo conditions at therapeutic concentrations of inhibitor. The H_2-receptor antagonists were not potent reversible inhibitors even when 10X therapeutic concentrations were modeled. There was evidence of time-dependent inhibition. This mechanism needs further study, as cimetidine is known to cause some significant in vivo drug interactions.

Keywords: Oxycodone, Methadone, Drug Interaction, Reversible Inhibition, H_2-Receptor Antagonists, Proton Pump Inhibitors

This research was supported by a grant from the National Institute of Justice, 2011-DN-BX-K532.

P31
Withdrawn

P32

Analytical Reference Standards: Synthesis and Characterization of Cocaine N-Oxide HCl

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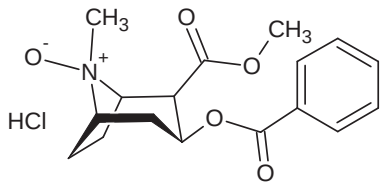
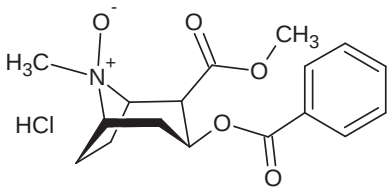
Introduction: Cocaine has been one of the most widespread illicit drugs of abuse in the US. Cocaine-N-oxide is a common metabolite found in hair of cocaine users. Cocaine N-oxide readily degrades to cocaine and norcocaine on GC/MS due to the compound's sensitivity to heat. Testing laboratories are developing LC/MS methods for direct screening and quantitation of this metabolite to monitor cocaine use in matrices such as hair. Certified Reference Materials (CRM) of high purity Cocaine N-oxide and its internal standard are required for accurate quantitation. Cocaine N-oxide is a mixture of equatorial and axial isomers.



Equatorial isomer of cocaine N-oxide



Axial isomer of cocaine N-oxide

Objective: To synthesize cocaine N-oxide HCl, characterize the isomers purity, and certify at a level suitable as a certified analytical reference standard in quantitative application. To determine the stability profile of the two isomers and identify the degradation product. To develop stable solution based CRMs of Cocaine N-odixe HCl and it's deuterated analog.

Method: Cocaine N-oxide HCl was synthesized from cocaine by oxidation with mCPBA. The identity of the two isomers was established through NMR and mass spectrometry. The chemical purity was established through HPLC/UV, Karl Fisher titration, GC/FID Headspace and inorganic content analysis. LC-MS/MS studies were performed on this compound to evaluate fragmentation patterns and suitability for use as an internal standand. The isomers were characterized by ^{1}H, ^{13}C and 2D NMR.

Results: During the synthesis, the free base cocaine N-oxide was found to be very unstable. It degraded to anhydroecgonine methyl ester N-oxide during reaction work-up. It was then isolated and purified as the HCl with purity of over 98%. The product contains two isomers of ratio of about 22/78. The two isomers were characterized by ^{1}H-NMR, ^{13}C-NMR and 2D NMR. The major isomer was determined to be the equatorial N-oxide. Rate of degradation was compared and correlated with the axial and equatorial isomer ratio.

Conclusion: Cocaine N-oxide HCl and Cocaine-D_3 N-oxide HCl were synthesized in over 98% purity and suitable for use as quantitative reference standards. The two isomers were characterized by HPLC and NMR. Degradation studies were needed to develop a solution based CRMs of cocaine N-oxide HCl and its deuterated internal standard.

Keywords: Analytical Reference Materials, Cocaine, Cocaine N-Oxide HCl

P33

Identification of Metabolites of AKB-48, a Novel Synthetic Cannabinoid, Using Human Hepatocytes and High Resolution Mass Spectrometry

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Introduction: Since scheduling of the first generation synthetic cannabinoids including JWH-018 and JWH-073 by federal authorities, many new synthetic cannabinoids became available. N-(1-adamantyl)-1-pentylindazole-3-carboxamide (AKB-48), also known as APINACA, was recently observed in Japanese herbal smoking blends. The National Forensic Laboratory Information System registered 443 reports of AKB-48 cases across 32 states of the US from March 2010 to January 2013 and the Drug Enforcement Administration listed AKB-48 as a schedule I drug. The high pharmacological and addictive potency of AKB-48 on cannabinoid receptors and difficulty in detecting the parent compound in urine specimens highlights the importance of its metabolite identification for pharmacokinetic, toxicological and screening studies in clinical and forensic settings.

Objective: To generate a complete metabolite profile of AKB-48 using cryopreserved human hepatocytes and high resolution accurate mass spectrometry.

Method: Ten µM of AKB-48 was incubated with cryopreserved human hepatocytes up to 3 h in an incubator maintained at 37°C. Reaction was stopped with an equal volume of acetonitrile after 0, 1 or 3 h. Samples were centrifuged at 15,000 g at 4°C and the supernatant (5x diluted) was injected onto the LC column. Data were acquired with a TripleTOF® mass spectrometer coupled with high performance liquid chromatography (HPLC). The TOF method full mass scan range was 100-800 m/z and product ion mass scan range was 60-800 m/z, respectively. Chromatography was performed on a Kinetex™ C18 XB column (100 x 2.1 mm; 2.6 µm) with 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) with a gradient flow rate of 0.3 mL/min. Column and autosampler temperatures were 40 and 4°C, respectively. Data were analyzed by MetabolitePilot™ software employing peak finding algorithms and data mining tools such as product ion, neutral loss and mass defect filters. Minimum threshold criteria were set to detect as many relevant metabolites as possible.

Results: Seventeen novel phase I and II AKB-48 metabolites were identified. Major metabolites were formed by monohydroxylation, dihydroxylation and trihydroxylation on the aliphatic adamantane ring or the *N*-pentyl side chain. Glucuronide conjugation of some mono and dihydroxylated metabolites also occurred. Oxidation and dihydroxylation on the adamantane ring and *N*-pentyl side chain forming a ketone also were observed. *N*-dealkylation was observed as a minor metabolite. The data suggest various biotransformations occurred, including sequential metabolism, although, there was only a moderate rate of disappearance of the parent. A correlation of metabolite-time profile between 1 and 3 h hepatocyte samples suggested greater metabolism (greater number of metabolites) after 3 compared to 1 h samples.

Conclusion: For the first time, a complete AKB-48 metabolic scheme based upon human hepatocyte data is presented. Spectra acquired from this human hepatocyte metabolism study can be incorporated into spectral libraries employed in clinical and forensic laboratory urine screening methods for detecting consumption of AKB-48.

Keywords: AKB-48, Human Hepatocytes, High Resolution Mass Spectrometry, Metabolism

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P34

Rapid Identification of RCS-8 Metabolites by Human Hepatocyte Incubation and High Resolution Mass Spectrometry

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Introduction: New synthetic cannabinoids continually enter the designer drug market, challenging clinical and forensic toxicologists to develop sensitive, specific, and up to date analytical methods. Rapid identification and incorporation of new parent compounds and metabolites, which are usually the only urinary targets, is crucial. As marketed compounds are either completely new or developed for basic research, there is a deficit of information on the drug's pharmacodynamics and pharmacokinetics preventing controlled administration studies in humans. Consequently, it is of paramount importance to find other strategies for identifying new designer drug metabolites. We present an approach for metabolite identification combining human hepatocyte incubation, high-resolution mass spectrometry (HRMS) and software-assisted data mining. Human hepatocytes are better suited for predicting actual metabolite profiles than human liver microsomes or *in vivo* rat metabolism studies as they contain human phase I and II enzymes in appropriate relative concentrations. HRMS offers distinct advantages over conventional LC-MS: 1) unexpected metabolites can be detected by data mining of accurate mass full scan spectra and 2) accurate mass measurements for molecular and fragment ions facilitate determination of elemental composition and substructure identification. Data mining software also assists in streamlining metabolite identification.

Objective: RCS-8, a scheduled phenylacetylindole synthetic cannabinoid, was utilized as a proof of concept to demonstrate that a combination of hepatocyte incubation and HRMS is a promising tool for rapid generation of comprehensive metabolite profiles of new designer drugs.

Method: 10 μM RCS-8 was incubated with pooled human hepatocytes. Samples were taken after 0, 1 and 3h incubation. After centrifugation and 1:4 dilution with mobile phase, samples were analyzed by HPLC coupled to a TripleTOF 5600 mass spectrometer (ABSCIEX). Analysis consisted of a TOF survey scan and information-dependent acquisition triggered product ion scans, with mass defect filtering and dynamic background subtraction. Subsequent data mining of the accurate mass full scan and product ion spectra was performed by MetabolitePilot software (version 1.5, ABSCIEX) with different data processing algorithms, such as mass defect, common product ion and neutral loss filtering.

Results: More than 20 RCS-8 metabolites were detected from mono- and di-oxidation, demethylation, and combinations of these biotransformations. Most metabolites were further glucuronidated, primarily as aromatic hydroxyl groups. The main oxidation sites were the cyclohexyl and phenyl rings, but never the indole moiety. No dealkylation of the cyclohexylethyl moiety was observed. Major metabolites were hydroxyphenyl RCS-8 glucuronide, a variety of hydroxycyclohexyl-hydroxyphenyl RCS-8 glucuronides, hydroxyphenyl RCS-8, as well as the demethyl-hydroxycyclohexyl RCS-8 glucuronide.

Conclusion: Our study is the first to define major urinary targets of RCS-8. We also showed that the combined use of hepatocyte incubation, HRMS and software-assisted data mining is useful for rapid metabolite identification, improving documentation of designer drug intake during forensic and clinical investigations. This approach can clarify the origin of metabolites when one or more parent synthetic cannabinoids could be the source. In the future, we plan to process newly emerging substances as rapidly as possible to provide human metabolism data to the community and to further evaluate these data with authentic specimens.

Keywords: RCS-8 Metabolism, Synthetic Cannabinoid, Human Hepatocyte Incubation, High Resolution Mass Spectrometry

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P35
Observations and Results from a Zolpidem Related Fatality

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Introduction: Zolpidem is a short-acting sedative hypnotic used for the short-term treatment of insomia. It is sold under the brand names Ambien, Ambien CR, Edluar, Intermezzo and Zolpimist. Zolpidem actions occur within 15 min following oral administration. It has a short half-life of 2-3 hrs and is converted to pharmacologically inactive metabolites. In cases of acute intoxication, adverse clinical symptoms include dizziness, amnesia, headache, nausea, tachycardia, ataxia, slurred speech, emesis, hallucinations and even death.

Objective: Several fatalities involving zolpidem intoxication have been reported, but in the majority of reported cases, it was detected in combination with other drugs. This case presents a suicide fatality in which zolpidem was the only drug detected by routine toxicology screens. The zolpidem concentration was very high as compared to other zolpidem- associated fatalities reported in the literature and, thus, presents relevant toxicity data.

Method: Routine screening of postmortem femoral blood was performed using Enzyme Multiplied Immunoassay Technique (EMIT) and liquid-liquid alkaline extraction followed by GC/MS. Quantitation of zolpidem was performed by an external laboratory using LC-MS/MS.

Results: The subject was a 93 year old male with a history of heart disease, hypertension, asthma and GERD. His prescription medications included digoxin, torsemide, potassium chloride, metoprolol, tamsulosin, warfarin, Nexium, atorvastatin and albuterol. The subject was unhappy about being moved to an assisted living facility a few months prior to the incident and about no longer being allowed to drive. He threatened several times to take the entire bottle of zolpidem at once. The subject was found unresponsive in his bed. A 90 count zolpidem bottle was located on a table next to his bed. The bottle was empty and 2 tablets were found on the floor that matched the zolpidem prescription. Zolpidem was the only drug detected and the femoral blood zolpidem concentration was determined to be 4000 ng/mL. Therapeutic plasma reference ranges have been reported ranging from 58-272 ng/mL (Baselt's 9th ed.).

Conclusion: The cause of death in this case was determined to be zolpidem overdose, and the manner of death was suicide. The elimination half-life for zolpidem may be significantly prolonged in the elderly (Baselt's 9th ed.). This may be partially responsible for the significantly high zolpidem concentration in the peripheral blood. Most of the earlier reported cases of zolpidem overdose involved other drugs. The current case involved only zolpidem. As this drug continues to be used in the treatment of sleep disorders, zolpidem toxicity data is becoming increasingly important in the investigation of both DUIs and fatalities.

Keywords: Zolpidem, Overdose, Fatality

P36
Observations and Results from Three Synthetic Cannabinoids Related Fatalities

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Introduction: Synthetic cannabinoids have acquired national attention as an alternative to natural cannabinoids. Literature indicates that smoking these synthetic drugs can present psychological symptoms such as excitability, nightmares, and panic attacks. Physiological symptoms include nausea, profuse sweating, tachycardia, dyspnea, xerostomia, sedation, and headaches. Despite the DEA's efforts to combat the spread of these synthetic drugs, they are increasingly becoming popular among teens and young adults.

Objective: We present the results of three recent and unrelated fatality cases involving synthetic cannabinoids.

Method: Routine drug screening of postmortem specimens was performed using Enzyme Multiplied Immunoassay Technique (EMIT) and liquid-liquid alkaline extraction followed by GC/MS. However, these combined general screening methods will not detect synthetic cannabinoids in postmortem blood and urine. As a result, all screening and quantitation of synthetic cannabinoids were performed by an external laboratory using LC-MS/MS.

Results:
Case 1: The subject was a 26 year old male who was found unresponsive in bed by his girlfriend. He was known to be a methamphetamine user but reportedly quit a few days prior to his death. The drug screen was negative for amphetamines but positive for cannabinoids. The femoral blood was positive for XLR-11.

Case 2: The subject was a 52 year old male who was found unresponsive in his bedroom. He was a known methamphetamine and marijuana user. In this case, methamphetamine, hydrocodone, chlorpheniramine as well as JWH-250, JWH-081, and JWH-122 were detected. The urine was positive for JWH-018 metabolites: JWH-018 N-(4-hydroxypentyl) and JWH-018 N-(5-hydroxypentyl). The concentrations of methamphetamine, hydrocodone, and JWH-250 were 294, 172, and 0.79 ng/mL respectively.

Case 3: The subject was a 35 year old female who was the unrestrained driver of a single vehicle accident. The subject was a chronic alcohol abuser. The femoral blood ethanol level was 143 mg/dL and the vitreous ethanol level was 146 mg/dL. Drugs detected include delta-9-THC, alprazolam, and the synthetic compound, XLR-11.

Conclusion: In case 1, autopsy did not indicate any anatomic cause of death. The cause of death was concluded to be synthetic cannabinoids intoxication and the manner of death was accidental. The cause of death in case 2 was determined to be a combination of methamphetamine, hydrocodone and synthetic cannabinoids intoxication and the manner of death accidental. The cause of death in case 3 was blunt force trauma with ethanol intoxication being a contributing factor. A commonality observed in all three cases is that all three subjects were chronic drug abusers. Although synthetic cannabinoids intoxication is not well understood as a causative factor in fatalities, the cases presented here suggest a contributory role of synthetic cannabinoids in fatalities involving multiple drug toxicities and/or trauma, as well as a potential primary role in causing death.

Keywords: Synthetic Cannabinoids, Fatality, JWH-250, JWH-081, JWH-122, XLR-11

P37

Novel Conversion of an Extraction Method to a Dilute and Shoot Procedure for the Quantitation of 16 Benzodiazepines in Human Urine

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Introduction: The testing of benzodiazepines in urine has typically required some form of extraction using solid-phase extraction, liquid-liquid extraction, or even supported liquid extraction in order to isolate the analytes of interest from matrix. Converting the method from a more complex extraction process to a simplified dilute-and-shoot process would provide significant advantages for the analytical laboratory.

Objective: To validate a dilute and shoot method for the analysis of benzodiazepines in human urine using HPLC/MS/MS and evaluate the cost advantages of applying that method to the analysis of patient samples.

Method: The urine sample (25 µL) was fortified with 25 µL of internal standard solution (50 ng/mL of each labeled compound as noted in the table below) and 200 µL of deionized water. A 25 µL aliquot of β-glucuronidase solution (10,000 units/mL) was added to each sample and vortexed for 30 seconds. The sample was incubated at 55 ^{o}C for two hours followed by further dilution with 800 µL of 50:50 0.1% formic acid in water:methanol. Pipetting was performed using a Tecan Evo Freedom automated pipetting station. Ultra-high performance liquid chromatographic (UPLC) separation is performed on a Restek Pinnacle DB Biphenyl 1.9 µm, 2.1 x 50 mm column. Mobile phase A was 0.1% formic acid in water. Mobile phase B was methanol. Detection is achieved using a Shimadzu Nexera UFLC system with an AB SCIEX API6500 QTrap mass spectrometer in the positive ion MS/MS mode. A total of 16 benzodiazepines and 11 internal standards (43 MRM transitions) were analyzed using scheduled MRM in 3.5 minutes. The following benzodiazepines were analyzed (those with labeled internal standards are marked with an asterisk):

7-Aminoclonazepam*	Halazepam
7-Aminonitrazepam	Hydroxyalprazolam*
7-Aminoflunitrazepam*	Oxazepam*
Hydroxytriazolam*	Lorazepam*
Hydroxymidazolam*	2-Hydroxyethylflurazepam*
Bromazepam	Temazepam*
Estazolam*	Diazepam
Lormetazepam	Nordiazepam*

Validation consisted of linearity, accuracy, precision, specificity, carryover, and interference studies.

Results: The method is accurate and reproducible from 20 ng/mL to 4000 ng/mL for all benzodiazepines analyzed except for hydroxytriazolam (2,000 ng/mL) and 2-hydroxyethylflurazepam (1,000 ng/mL). Imprecision (calculated as %CV) for all analytes is less than 10% across the linear range of the method. Mean accuracy was calculated to be no more than 10% from nominal for all concentrations assessed except at the limit of quantitation (LOQ) and upper limit of linearity (ULOL) which was calculated to be no more than 20% from nominal.

In comparison to the previously validated supported liquid extraction method, the cost of sample preparation has been greatly reduced. Per batch, the cost of sample preparation (materials and labor) has decreased by approximately 80%. The time necessary for sample preparation has been reduced by approximately one hour for each batch. No reduction in column lifetime has been observed compared to the previous method with some columns lasting more than two thousand injections.

Conclusion: A quantitative dilute-and-shoot LC/MS/MS method has been validated for the determination of benzodiazepines in urine. The method is precise and accurate and is currently used in production for donor sample analysis. Implementing this new methodology has allowed for significant material and labor cost savings compared to previously employed methodologies.

Keywords: Benzodiazepines, Quantitative Dilute-and-Shoot LC/MS/MS

P38
Tissue Distribution of Isoflurane After Lethal Intoxication

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Introduction: Isoflurane, 1-chloro-2, 2, 2,-trifluoroethyl difluoromethyl ether, is a volatile anesthetic used for general anesthesia in animals under veterinary surgical care. The adverse effects of inhaling isoflurane include respiratory depression, hypotension, cardiac arrthymias, nausea, and vomiting.

Case Histories:

- Case #1: A 53 year old professor, with a doctorate in neuroscience, was found decomposed in his home. The decedent's body was wrapped in plastic with multiple bottles of isoflurane on the scene, along with containers of alcohol.
- Case #2: A 44 year old anesthesiologist was found expired in his home along with several empty bottles of isoflurane and a sock with a chemical smell. He had a history of substance abuse and depression.

Objective: 1. To validate a method for isoflurane analysis using routine headspace gas chromatography with flame ionization detection, that will allow separation, detection, and quantification of isoflurane in different biological matrices. 2. To characterize the postmortem distribution of isoflurane in an isoflurane intoxicated individual.

Method: Isoflurane was obtained from JD Medical. Specimens and internal standard were aliquoted into crimp-sealed headspace vials using a Hamilton pipettor-diluter. Separation of isoflurane and other volatiles was achieved with Restek, Rtx®-BAC1 and a Rtx®-BAC2, capillary columns using an Agilent 6890 Plus GC equipped with dual FID and a HP-7694 Headspace Auto-sampler. Confirmation was performed by headspace gas chromatography mass spectrometric detection using an Agilent 6890 GC/5975 MS-EI in full scan mode using a Rtx®-BAC1 column.

Results: Case #1: An autopsy was performed at the Cuyahoga County Medical Examiner's Office, Cleveland, Ohio. The body was a normally developed, middle aged, white male with cutaneous and visceral changes of decomposition, fluid accumulation in the thoracic cavity, and microscopic evidence of hepatic steatosis.

The concentrations of isoflurane in tissues for Case #1 are shown in the table below.

Specimen Concentration (µg/mL)	Thoracic Cavity Fluid	Femoral Blood	Brain	Gastric Contents	Bile	Muscle	Lung	Liver
	5	34	54.5	33µg/125mL	7	9	9	102

Other blood and tissue results for Case #1 were as follows:

Specimen Concentration (µg/mL)	Thoracic Cavity Fluid	Femoral Blood	Brain	Gastric Contents	Bile	Muscle	Lung	Liver
Ethanol (g/dL)	0.16	0.087	0.044	0.07	0.046	0.121	0.093	0.077
Acetaldehyde	Pos	-	-	Pos	Pos	-	-	-
Acetone	-	-	-	Trace	-	-	-	-
Ethyl Acetate	Trace	-	-	Trace	-	-	Trace	Trace
1-Propanol	Trace	-	Trace	Trace	-	Trace	Pos	Trace
1-Butanol	-	-	-	-	-	Pos	-	-

Case #2: An autopsy with toxicology testing was performed at the Office of Chief Medical Examiner, New York, New York. Other than slight generalized visceral congestion, no findings of note were observed. There were no stigmata of intravenous drug abuse. The concentration of isoflurane was analyzed only in the cardiac blood by NMS Labs; < 3 µg/mL of isoflurane was detected. No other volatiles were found. Comprehensive toxicology screens were performed on cardiac blood, femoral blood, and urine; no other drugs were detected.

Conclusion: Case #1: Isoflurane was distributed throughout the tissues with values ranging from 5.0-102 µg/mL. This finding supported the ruling of an acute isoflurane and ethanol intoxication. The death was ruled an accident.
Case #2: The death was ruled an accidental acute isoflurane intoxication.

Keywords: Isoflurane, Volatiles, Anesthetic

P39

Evaluation of the Avoximeter-4000® to Measure the Stability of Carboxyhemoglobin in Different Blood Collection Tubes Over Time

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Introduction: Carboxyhemoglobin (COHb) is typically monitored by toxicology laboratories in persons who die in house fires or are found dead in automobiles. Carbon monoxide (CO) displaces oxygen bound to hemoglobin with a binding affinity approximately 300 times that of oxygen. As a result, even low levels of CO for extended periods of exposure will result in toxic to lethal levels of COHb. At Cuyahoga County Medical Examiner's Office, COHb is determined using an Avoximeter 4000® (International Technidyne Corporation) with confirmations performed spectrophotometrically. The Avoximeter is a CO-oximeter which uses disposable/fillable cuvettes. There is no tubing or pump to malfunction and the analysis is complete in < 1 min.

Objective: The current study evaluated the Avoximeter and the stability of COHb levels in different blood collection tubes routinely used to collect specimens during autopsy. Included were gray top (NaF and potassium oxalate) red top (no additives), purple top (EDTA), green top (heparin), and blue top tubes (acid washed-no additives).

Method: A unit of red cells and a unit of plasma were obtained from University Hospitals in Cleveland. Both units had expired and were to be discarded. The red cells and plasma were combined to make whole blood. Sodium dithionite (500 mg) was added to convert hemoglobin to deoxyhemoglobin. CO gas (99.5%) was then bubbled through the whole blood to convert hemoglobin to carboxyhemoglobin; Dissolved CO was removed from solution by bubbling with air for an extended period. Carboxyhemoglobin was monitored using an Avoximeter. The level of COHb for time zero was adjusted to 69.8 % by addition of oxyhemoglobin. The Avoximeter has an upper limit of measure of 75 % COHb. Confirmation of COHb concentration was made spectrophotometrically by the method of Rodkey. Blood was dispensed into five replicates of the different blood tubes and the level of COHb measured for 14 consecutive days. Evaluation of statistical differences was by single factor analysis of variance using an Excel® spreadsheet.

Results: Levels of COHb in red, green, purple and blue blood collection tubes remained essentially unchanged from Time 0 during the 14 day period (range 67.2 to 71.4 %). The %CV ranged from 1.12 to 1.97. COHb levels in gray top collection tubes were significantly lower ($p < 0.05$) with a mean of 63.6 % and %CV = 2.80. Results of the control procedure measured 69.2 % COHb which confirmed the accuracy of the Avoximeter.

Conclusion: The results of this study confirm earlier findings of Kunsman, et.al., 2000 demonstrating the stability of COHb in blood collection tubes stored under refrigerated conditions. Why gray top collection tubes produced a somewhat lower value is unknown at this time. No interactions of NaF or potassium oxalate with COHb have been reported. This study shows that measurement of COHb from different types of blood tubes is an acceptable practice. The Avoximeter was found to be accurate, precise, quick and extremely easy to use. The stability of COHb will continue to be tracked in the different blood collection tubes for even longer periods of time.

Keywords: Avoximeter, Carboxyhemoglobin, Stability

P40

New Concerns for an "Old" Drug? Dihydrocodeine: Two Fatalities in Northeast Ohio

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Introduction: Dihydrocodeine (6-alpha-hydrocodol, DHC) is an opioid agonist, prescribed for moderate to severe pain. In European countries DHC has been used to treat opioid addiction in place of methadone or buprenorphine. In the US, DHC is formulated with caffeine and acetaminophen (APAP) or aspirin (i.e. Synalgos, Panlor), but is not available as an isolated drug. Until recently, detection of DHC at The Cuyahoga County Medical Examiner's Office (CCMEO) has only been attributed to hydrocodone (HC) metabolism. This study describes two cases of DHC toxicity which are not consistent with HC exposure.

Case Histories:

- **Case #1:** A 51 year old male, reported as a sudden death at home, with a previous stroke history and prescriptions for Nucynta® (Tapentadol) and morphine for chronic pain, and also medications for hypertension and depression.
- **Case #2:** A 56 year old male, found deceased at home on the bathroom floor. He was known to abuse prescription pills.

Objective: To inform toxicologists of two recent deaths related to possible DHC intoxications, and the need to include this analyte in opiate procedures.

Method: Specimens collected at autopsy were kept refrigerated until analyzed. Blood samples were screened using ELISA (Immunalysis® Opiates Direct Kit 207-480). Extraction of DHC was by solid phase extraction (UCT Clean Screen® ZSDAU020) followed by derivatization with MSTFA (UCT). Analytes were separated, detected and quantified using an Agilent GC/EI-MS in the SIM mode with a Restek Rxi®-5ms, 30 m X 0.25 mm i.d., 0.25 µm film thickness, analytical column. Toxicological analyses were also performed by outside reference laboratories for drugs other than DHC.

Results: Table 1. Concentration of DHC detected in specimens from two postmortem cases.

Case	Heart Blood (ng/mL)	Femoral Blood (ng/mL)	Liver (ng/g)	Vitreous Humor (ng/mL)	Urine (qualititative)	Bile (qualitative)
Case #1	**238**	**277**	**448**	**270**	**Positive**	**Positive**
Case #2	**1951**	**1160**	**1824**	**1454**	**Positive**	**Positive**

Additional Toxicology:

- **Case #1:** Femoral blood contained 1447 ng/mL codeine, 125 ng/mL morphine, 32 ng/mL hydrocodone*, 18 ng/mL cyclobenzaprine*, 1500 ng/mL citalopram*, 96 ng/mL bupropion*, 740 ng/mL hydroxybupropion*, 350 ng/mL tapentadol**, 33 ng/mL gabapentin**, 507 ng/mL atenolol**, 110 ng/mL hydrochlorothiazide**, 45 mg/L APAP and caffeine positive.
- **Case #2:** Femoral blood contained 12 ng/mL hydrocodone, 80 ng/mL alprazolam and bromazepam positive**.

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Conclusion: Prior to these cases, DHC has been detected in many cases at CCMEO, but only in conjunction with HC. Although both of these cases contain HC, the ratio of DHC to HC is not consistent with HC usage. DHC:HC ratios resulting from HC exposure are typically < 0.5. The ratio of DHC to HC in femoral blood for these two cases was 8.6 and 96.6, respectively. A five year retrospective analysis of a CCMEO database revealed 75 cases in which the average femoral blood DHC:HC ratio was 0.26 ± 0.23. Similar results were found by Franklin County Coroner's Office, Columbus, Ohio, with an average femoral DHC:HC ratio of 0.31 ±0.23 from 22 cases during the past three years. In no cases previously observed was the DHC:HC ratio > 2. DHC was considered to be a contributing factor of death in both cases presented in this study. Previous reports indicate DHC intoxications are fatal at concentrations ranging from 1900 ng/mL-12,000 ng/mL. Detection of caffeine and APAP in Case #1 supports the possibility that the decedent had been prescribed DHC in the US (although case history does not include a prescription). A pill slurry collected from the gastric contents of Case #2 tested positive for DHC with no HC; absence of any other analytes suggests the DHC consumed may have been purchased outside of the US or over the Internet. The observation of two fatalities at CCMEO in which DHC seemingly played a role in cause of death demonstrates the need for laboratories to include DHC in their opiate procedures.

Keywords: Dihydrocodeine, Opiates, Postmortem, United States

P41

Rapid Point-of-Collection Testing for Δ^9-Tetrahydrocannabinol in Oral Fluid

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Introduction: Driving under the influence of alcohol (DUI or DWI) has been long recognized as impairing ones ability to drive and law enforcement having stopped drivers for suspicion of DUI, have been able to test, and thus enforce laws against DUI. On the other hand, driving under the influence of drugs (DUID) has only recently gained attention as a danger practice. The National Highway Traffic Safety Administration found that in 2009, among fatally injured drivers, 18 percent tested positive for at least one drug (e.g., illicit, prescription, or over-the-counter), which represents an increase from 13 percent in 2005. With the recent changes in laws allowing for the usage of marijuana, either recreationally or medicinally, this number is projected is further increase in next few years.

Objective: To begin to address these concerns, we have engineered a point-of-collection, robust, presumptive test for the presence of Δ9-tetrahydrocannabinol (THC) in saliva. Typical marijuana smokers experience a high that lasts approximately 2 hours and most behavioral and physiological effects return to baseline levels within 3-5 hours after drug use. Recent studies have determined that the half-life of THC in saliva ranges from 11.5 min to 31.1 min with a mean of 20.8 min and thus THC in saliva would provide the good matrix for a strong correlation between impairment and cannabis consumption.

Method: The platform is based on the traditional sandwich lateral flow assay architecture, and the test is capable of sensing low concentrations of THC direct from saliva of 10 ng/mL – in line with current laws in Colorado and Washington. The sample first encounters gold nanoparticles, which are labeled with antibodies against THC, then the test line is spotted with antibodies to the THC and the control line is spotted with THC.

Results: The time required from sample collection to read-out is less than 15 minutes, which is a reasonable time for law enforcement to determine the roadside sobriety of the driver.

Conclusion: It should be noted that there is currently no roadside THC test to correlate impairment with marijuana usage. The currently methodology for testing for THC usage is spectroscopic analysis of either urine or blood. In these test, the secondary metabolites, THC-COOH (or carboxy-THC) is quantified and then correlated to usage and time of impairment. As stated above, typically a high last approximately 2 hrs. and thus the times from arrest to test will not always accurately indicate drive under the influence of drugs. Our current work is focused on validating the lateral flow assay versus these current detection methods - GC/MS detection of carboxy-THC.

Keywords: Point of Collection Test, Driving Under the Influence of Drugs, Lateral Flow Assay

P42

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

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Introduction: The LC-QTOF-MS has been suggested to be a powerful screening tool for designer drugs in different forensic science settings due to its high resolution, high mass accuracy and MS/MS capabilities. This enables higher confidence in the identification of compounds such as designer drugs in complex biological matrices. In order to achieve the full screening potential of the LC-QTOF-MS for designer drugs, a high resolution MS/MS designer drug spectral library and a compound database were created.

Objective: The purpose of this project was to develop and test the applicability of a high resolution MS/MS spectral library and a compound database that contains designer drugs from multiple designer drug classes using LC-QTOF-MS instrumentation.

Method: An Agilent 1290 Infinity HPLC system with a 6530 Accurate-Mass Q-TOF LC-MS with an Agilent Jet Stream Technology electrospray ion source was used for this project. Drug standards were directly injected 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 90:10 acetonitrile:water with 0.1% formic acid. MS/MS spectral data was collected at three different collision cell energies (10 eV, 20 eV, and 40 eV). Creation of the compound database entailed identifying designer drugs from various sources. For each designer drug in the database information such as compound name, chemical formula, monoisotopic mass, chemical structure and IUPAC name was added to the compound database. The spectral library and database were created using MassHunter PCDL Manager software. A qualitative screening method was created using a cation mixed mode extraction cartridge (Agilent Bond Elut Plexa PCX, 60 mg) for the extraction and the Agilent Zorbax Rapid Resolution HD Eclipse Plus C_{18} column (3.0 x 100 mm 1.8 micron particle size) for the separation. Spiked water and serum samples were used to determine the applicability of the library and database.

Results: The high resolution MS/MS spectral library includes data from 263 designer drug standards. In addition to the spectral library, the compound database contains information for approximately 550 additional designer drug compounds. This information can be used to help aid in the identification of designer drugs in full mass scan mode. A qualitative screening method was developed for 35 designer drugs from various classes. LOD's were <10 ng/mL for most analytes. The library was able to identify designer drugs in spiked water and serum matrices.

Conclusion: A designer drug high resolution MS/MS spectral library and compound database were created in order to enhance the screening potential of the LC-QTOF-MS. A qualitative method that can screen for multiple designer drug classes was created to successfully test the library and database. The combination of a high resolution MS/MS library and a compound database could be a useful tool for the identification of designer drugs in forensic toxicology screening applications.

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

P43

Determination of 15 Antiepileptic Drugs and Two Selected Metabolites in Post-Mortem Whole Blood Using LC/MS/MS

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Introduction: In recent years, there has been a growth in reports of antiepileptic drugs (AEDs) being misused on their own or in combination with other drugs of abuse such as heroin and other opioids in a variety of toxicological case types such as drug abuse, suicide, overdose and drug facilitated crime. Some of these AEDs are part of routine forensic analysis e.g. carbamazepine and phenytoin however many are not. A number of laboratory methods have been described to quantify AEDs and their metabolites but all of these are for therapeutic drug monitoring purposes or for one drug and its metabolites. To our knowledge, there are no simultaneous quantification methods for the simultaneous analysis of the most commonly encountered AEDs in post mortem whole blood to include lacosamide, eslicarbazepine and retigabine in addition to gabapentin, pregabalin, topiramate, tigabine, lamotrigine, zonisamide, valproic acid, levetiracetam, vigabatrin, oxcarbazepine, carbamazepine and its metabolite carbamazepine-10, 11-epoxide, phenytoin and its metabolite 5-(p-hydroxyphenyl)-5-phenylhydantoin (p-HPPH).

Objective: The aim of this project was to develop and validate a method for the determination of 15 AEDs and two metabolites in whole blood using liquid chromatography triple quadruple tandem mass spectrometry (LC/MS/MS) which would be suitable for routine forensic toxicological analysis.

Method: A 100 µl aliquot of the sample was transferred to a 2-mL snap top polypropylene microcentrifuge tube, 50 µL of internal standards solution (Gabapentin–D10, Tolbutamide and 10, 11 dihydrocarbamazepine) and 250 µL methanol were added, vortexed for 30 seconds and centrifuged for 10 minutes at 5000 rpm. An aliquot of 200 µL of the supernatant was transferred to LC vial and diluted with 1.5 mL of deionized water. A 5 µL of the diluted supernatant was injected into an Agilent LC/MS/MS 6420 triple quadruple system coupled with an Agilent 1200-series LC system. Electrospray ionization (ESI) was used in dynamic multiple reaction monitoring mode with ion mode switching. The column used was a Gemini Phenomenex –C18 (150 mm x 2.1 mm, 5 µm). Gradient elution was chosen using a mobile phase consisting of 2mM ammonium acetate and methanol at a flow rate of 0.3 mL/min. The total run time was 20 minutes with a column temperature maintained at 40 °C.

Results: All AEDs were detected and quantified within 20 minutes without endogenous interferences. The linear range for each AED was as follows: valproic acid, vigabatrin and levetiracetam: 5-300.0 mg/L; carbamazepine, carbamazepine-10, 11-epoxide, eslicarbazepine, topiramate, lamotrigine, lacosamide, pregabalin and gabapentin: 0.5-50 mg/L; zonisamide, phenytoin and its metabolite p-HPPH: 1-50 mg/L. Oxcarbazepine and tigabine 0.05-10 mg/L; retigabine: 0.5-10 mg/L. The correlation coefficient (R2) was greater than 0.994 for all AEDs with accuracy and precision exceeding 85% ±15% for all analytes. The recovery ranged from 50% to 98%. No carryover was observed in a blank injected after the highest standard and the matrix effect was acceptable and ranged from 90% to 120%.

Conclusion: A simple, accurate, and sensitive LC-MS/MS method has been developed for the simultaneous quantification of 15 AEDs and 2 metabolites. The method has been used for routine toxicological analysis of post-mortem cases.

Keywords: Antiepileptic Drugs, Drug Abuse, LC/MS/MS, Post-Mortem Blood

P44

Storage and Transportation Studies of Amphetamine, Methamphetamine, Benzoylecgonine, Phencyclidine, Morphine, Oxycodone, and Δ9- Tetrahydrocannabinol in the Quantisal™ Collection Device

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Introduction: An investigation into the stability of drugs in oral fluid using the Quantisal™ collection device during normal storage and transportation conditions was carried out. Three separate experiments were conducted; stability at room temperature, stability at refrigerated temperature, and overnight transportation.

Objective: The objective was to determine optimal conditions for sample handling for both collector and laboratory. Drug concentration was determined by LC-MS/MS for amphetamine (AMP), methamphetamine (METH), benzoylecgonine (BZE), phencyclidine (PCP), morphine (MOR), and oxycodone (OXYC), and GC-MS for Δ9-tetrahydrocannabinol (THC). All methods are fully validated and tested against both proficiency and real user specimens.

Method: 50mL of neat oral fluid was collected, pooled, and divided into two 25mL aliquots. Multi-analyte drug solutions were prepared at -50% (solution A) and +50% (solution B) of the cutoff for AMP (50ng/mL), METH (50ng/mL), BZE (30ng/mL), PCP (10ng/mL), MOR 30ng/mL), OXYC (30ng/mL), and THC (4ng/mL). 16 collection pads soaked up 1mL of fortified oral fluid for each level. When the collection pad indicator turned blue each pad was placed into a corresponding labeled tube. Samples were stored at room temperature in the dark and analyzed in duplicate at time points 0, 7, 14, and 30 days. Samples were stored at 4°C and analyzed in duplicate at time points 14 and 30 days. After analysis of time zero, specimens replicate were packed in a thermo container which included temperature and humidity logs and shipped overnight to the East coast and back to the West coast for analysis.

Results: An analytical result within +-20% of the original value (day zero) was considered to be an acceptable analytical variable. All drugs except THC were within this range up to day 30 when stored at room temperature in the dark. THC showed a 30% and 25% loss for solutions A and B respectively by day 7 with a 60% and 30% loss by day 30. At refrigerated temperature (4°C) all drugs at all times points for both solutions A and B showed minor degradation and were within +-20% range. THC showed a 10% and 2% loss for solutions A and B by day 30 when stored at refrigerated temperature. The temperature during overnight transportation ranged from 14.8 – 30.1°C and no significant loss was seen for any drug. The low concentration THC solution had a minor loss of 10% while the +50% solution had a loss of 3%.

Conclusion: Drugs are stable within the Quantisal™ oral fluid collection device when shipped overnight without cold packs in regulation containers. Once received into the laboratory the Quantisal™ device should be stored refrigerated at 4°C especially if THC analysis is required. Samples should be analyzed within the first week of receiving but can be stored for at least 30 days without significant loss. This has proven to be helpful for re-analysis purposes.

Keywords: Oral Fluid, Stability, Storage

P45

Drug Stability in Authentic Oral Fluid Specimens Collected with the Quantisal™ Device

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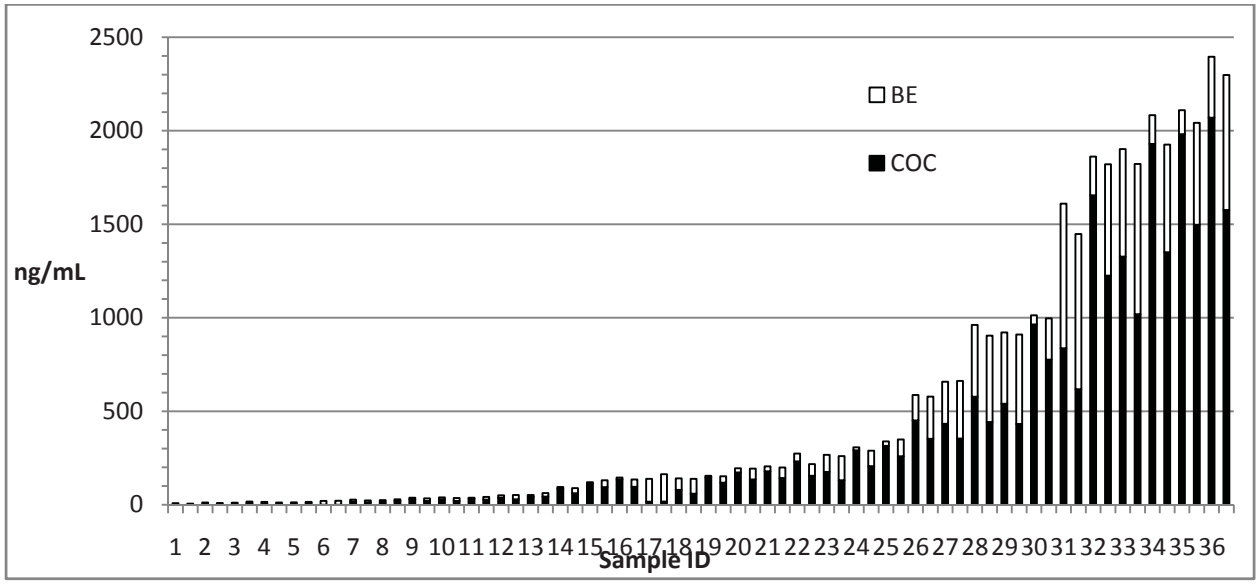
Introduction: An investigation into the stability of drugs in oral fluid collected with the Quantisal™ device was conducted. Specimens routinely received into the laboratory were analyzed, then decanted and stored in borosilicate glass tubes in refrigerated conditions (4°C). After 3 months, the specimens were re-analyzed for drugs which had originally confirmed. Analysis included amphetamine (AMP), methamphetamine (METH), MDMA, MDA, cocaine (COC), benzoylecgonine (BZE), cocaethylene (CE), Δ9-THC, morphine (MOR), hydrocodone (HYC), oxycodone (OXYC), ketamine (KET), methadone (MTD), and phencyclidine (PCP).

Objective: The objective was to determine the stability of drugs in oral fluid in the Quantisal™ collection buffer. No sample contained any bacterial growth or noticeable decomposition.

Method: All LC-MS/MS and GC-MS analytical methods were fully validated in accordance with published guidelines. GC-MS was used for THC and PCP analysis and LC-MS/MS was used for all other drugs.

Results: The average change in concentration for all drugs, including THC was less than +/- 20% except for COC and BZE.

- Cocaine: COC had an average loss of 24% (n = 36), degrading as the BZE concentration increased significantly (average gain: 150%). One cocaine positive sample dropped from 8ng/mL to 6ng/mL with no measurable BZE reported during either analysis. The figure below shows a side-by-side comparison of original and 3 month results for authentic specimens. The total concentration does not change while the ratio of BZE to COC does.



- THC: All samples analyzed for Δ9-THC (n=96) after 3 months still confirmed positively; Δ9-THC had a nominal average gain of 5.7%.
- AMPS: All reconfirmations for the amphetamine class were within +-20% of the original value: AMP (n=17) had an average 10% loss; METH (n=20) averaged a 2% gain; MDMA (n=23) was essentially unchanged and MDA (n = 6) showed a slight increase of 8%.
- Opiates: Opiate concentrations as well as ketamine, methadone and PCP were unchanged over the 3 months of storage

Conclusion: All original positive samples (n=217) (except one low level COC) remained positive after 3 months. Drugs present in authentic oral fluid samples collected with the Quantisal™ device, and stored in transportation buffer in glass vials at 4°C, are stable for at least 3 months.

Keywords: Oral Fluid, Stability, Storage

P46

Discrimination of Single and Repeated Xylazine Exposure in Skeletal Tissues Using LC-TOF-MS

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Introduction: Interpretation of toxicological measurements in bone is complex due to an absence of reference databases, standard methods and calibration challenges. Studies using controlled exposures in laboratory animals provides insight on variables influencing interpretation of measurements of drugs and metabolites in bone.

Objective: This work was done to determine if two different patterns of xylazine exposure could be discriminated through measurement of xylazine (XYL) and 4-hydroxyxylazine (4OH-XYL) levels; or the ratio of levels of 4OH-XYL and XYL (i.e.; 4OH-XYL/XYL) in decomposed bone.

Method: Twelve male Wistar rats were divided into two groups; with five rats per dose condition and two drug-free control animals. The first group (ACU) received one injection (45 mg XYL/kg i.p.) and were sacrificed by CO_2 asphyxiation 15 minutes post-dose. The second group (repeated dose - REP) received three injections (15 mg XYL/kg each; i.p.) and were sacrificed 40 minutes after the third dose. Remains decomposed to skeleton outdoors in Northern Ontario in secure cages for 3 weeks. Skull, vertebrae, ribs, pelvi and femora were recovered from each group, and were rinsed lightly with methanol; phosphate buffer (PBS: 0.1 M; pH 6) and acetone (3 mL each) and air dried overnight. Bones were ground using a domestic grinder. Samples of each bone (0.2 g) were then extracted in methanol (10 mL; 72 hrs; 50 ^{o}C). Extracts were recovered; evaporated and reconstituted in 1ml PBS. Internal standard (D3-XYL; 600 ng); 100 µL glacial acetic acid and acetonitrile:methanol (1:1; 3 mL) was added to each extract; followed by storage at -20 ^{o}C overnight. Following centrifugation; supernatants were evaporated to 1 ml; and diluted with 3 mL PBS. Diluted supernatants underwent mixed-mode SPE. Following column conditioning and sample loading, columns were washed with PBS (3ml) and 0.1 M acetic acid (3 mL); and dried (~ 5 in Hg; 5min). Columns were then washed with methanol (3 mL) and dried again under vacuum (~10 in Hg; 10 min). Elution was done with 3% NH_4OH in 20:80 isopropanol:ethyl acetate (6 mL). Extracts were evaporated and reconstituted in 0.1% formic acid in 10:90 acetonitrile:water (350 µL), followed by analysis by LC-TOF-MS. XYL and 4-OHXYL levels were measured as the mass-normalized response ratios (RR/m), using extracted ion profiles (m/z 221.103, 237.303 and 227.303 for XYL, 4OHXYL and D3-XYL, respectively. Drug levels and 4OH-XYL/XYL ratios were compared using Student's t-test, with significance difference attributed where $p < 0.05$. This method is linear from 10-2000 ng/mL in each analyte, with limit of detection of 5 ng/mL and precision of replicate measurements over the linear range < 20% (n = 5).

Results: Levels (RR/m) of XYL differed significantly between treatments, while levels of 4OH-XYL did not, except in skull, where levels of 4OH-XYL differed significantly. However, in all bones except rib, values of (4OH-XYL/XYL) differed significantly between exposures. When results were pooled across all bone types, XYL levels differed significantly ($p < 0.001$) between exposure patterns; while 4OH-XYL levels did not ($p = 0.63$). However, values of 4OHXYL/XYL differed significantly between exposure patterns ($p < 0.001$). XYL concentrations ranged from approximately 0.67-11 and 0.08-3.8 µg/g in the ACU and REP groups respectively, while 4OH-XYL concentrations ranged from approximately 0.09-1.3 and 0.06-1.0 µg/g in the ACU and REP groups, respectively.

Conclusion: The exposures examined here are best discriminated by XYL levels or the ratio of responses of metabolite and parent (4OHXYL/XYL). The latter may be the preferred metric since it better addresses challenges in absolute drug quantitation in bone.

Keywords: Bone, Xylazine, LC/MS

P47

Analysis of Pentobarbital in Decomposed Bone by Microwave-Assisted Extraction and Gas Chromatography-Mass Spectrometry

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Introduction: Bone remains one of the most poorly characterized analytical matrices in forensic toxicology.

Objective: Passive solvent extraction has been used for sample preparation of bone tissue samples, but requires extensive incubations, thus limiting analytical throughput. Accordingly, methodologies for rapid microwave-assisted-extraction (MAE) of drugs from decomposed bone tissue are in development. In this work we describe development of MAE of pentobarbital from porcine bone tissue.

Method: Bone was derived from remains of a domestic pig exposed to diazepam (7.5 mg/kg), citalopram (7 mg/kg), amitriptyline (75 mg/kg), morphine (0.8 mg/kg) and euthanized with an injection of PB (30 mg, IP) followed by a lethal intracardiac injection of PB (390 mg). After euthanasia, remains decomposed to skeleton outdoors (rural Ohio) for over 2 years. Bones were recovered and separated according to anatomic site. Bones were lightly rinsed with phosphate buffer (PBS, 0.1 M, pH 6), methanol and acetone and dried under ambient conditions, and then pulverized with a domestic grinder. Porcine bone assayed to yield no detectable pentobarbital was used as a negative control. Ground vertebrae were sampled in triplicate and the effects of sample mass (1-2 g), solvent polarity (methanol vs. ethyl acetate), solvent volume (5, 10 and 15 mL) and extraction time were investigated with respect to analyte yield. Samples were extracted for prescribed periods of time, followed by solvent removal and replacement with fresh solvent 3, 6, 9, 15, 20, 30 min for microwave irradiation. Samples were evaporated to 1 mL and the volume was then adjusted to 4 mL with PBS. Secobarbital (SB, 50 ng) was added as an internal standard. Lipid/protein precipitation with 1:1 acetonitrile: methanol (3 mL) preceded mixed-mode solid phase extraction (SPE). Following conditioning and sample loading, columns were washed sequentially with PBS and 0.1 M acetic acid before PB was eluted with 3 mL of methanol. Extracts were evaporated to dryness and reconstituted in 50 µL ethyl acetate (EA) and 50 µL derivatizing agent (TMPAH) before analysis. GC/MS-SIM analysis monitored ions at m/z 112, **169** and 184 (PB) and 181, 195 and **196** (SB). The limit of detection was approximately 5 ng/mL PB (based on 1 mL initial solution), with linear response over 10-2000 ng/mL, and precision of replicate measurements < 20%. Mass-normalized response ratio (RR/m) was used for all statistical analysis (ANOVA one-way with post-hoc testing) in comparing relative drug yield in all bone tissue extracts.

Results: Methanol provided significantly higher extraction yields than ethyl acetate and solvent volume did not significantly influence analyte yield. MAE was rapid, with maximum extraction achieved after approximately 15 min.

Conclusion: MAE is rapid method for preparation of bone samples for toxicological drug screening. The method may be beneficial for rapid drug extraction from bone in cases of death investigation involving human skeletal remains.

Keywords: Bone, Pentobarbital, GC/MS, Microwave-Assisted Extraction

P48

Estimation of the Influence of "Dust-Off" Gases on Breath Alcohol Measurements Using the Intoxilyzer 8000C

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Introduction: The Intoxilyzer 8000C, a breath alcohol analysis instrument used in Canada, utilizes combined infrared absorbance measurements at both 3.4 and 9.4 μm wavelengths to give a measure of equivalent blood alcohol content (BAC), using the 2100:1 blood-breath ratio and a breath alcohol concentration (BrAC). As previously explored, this instrument has the potential to be affected by other forensically relevant substances that may elevate apparent BAC values.

Objective: This study was done to examine the potential for interference in BrAC measurements by "Dust Off®", a compressed gas containing 1,1-dilfluroethane (1,1-DFE) as well as a bittering agent, using the Intoxilyzer 8000C. Postmortem cases involving 1,1-DFE have had associated blood concentrations in excess of 21 mg/L [1], while occupational health studies involving passive exposure resulted in blood 1,1-DFE concentrations under 2.6 mg/L [2]

Method: "Dust Off®" was sprayed directly into degassed distilled water in the wet bath simulator. Solutions were prepared at concentrations of 2, 4, 20, 40 and 100mg/L total dissolved gases, weighed by difference into the degassed water. Mass equilibrium was achieved, and target concentrations of total added gas were obtained by diluting the solution to a final desired concentration. At levels of 20mg/L or less, replicates of 20 samples from the headspace vapor (34 ± 0.2 °C) were analyzed in Stability Check mode, and both "Interferent Detect" and "Range Exceeded" messages were noted on the Intoxilyzer 8000C. The last apparent BAC value prior to either warning message was also recorded.

Results: "Range exceeded" messages were observed in trials of 100, 40, and 20mg/L, with last apparent BrAC values of 317-747 mg/210L, 759-957 mg/210L and 568-599 mg/210L respectively. The "Range exceeded" message continued for at least five trials at initial added gas concentrations of 100 and 40mg/L, prompting a discontinuation of the examination. "Interferent detect" messages were observed in trials of 20, 4, and 2mg/L with last apparent BAC values of 551 mg/210L, 62 and 27 mg/210L, respectively, after the first test. In trials for the 20, 4, and 2mg/L initial added gas concentrations, no error messages were observed once the apparent BrAC reached 16 mg/210L.

Conclusion: Direct examination of the contents of "Dust Off®" cans is preferable to analysis of difluorethane standards since "Dust Off®" is a mixture of multiple components, with different vapour-liquid equilibrium behavior. However, the contribution of each component to the interferences observed is unclear. The maximum apparent BrAC overestimation in the absence of an "interferent detect" message was determined to be 16 mg/210L, and was observed after 13 and 6 calibration checks (4 and 2mg/L initial added gas concentrations, respectively). These findings should be considered when assessing cases of suspected DUI. These results also underscore the need for extended observation/deprivation periods.

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Keywords: "Dust Off®", 1,1-Difluoroethane, Intoxilyzer, Intereferent

P49

General Unknown Screening in Postmortem Blood Specimens by UHPLC-QTOF/MS and Automated Library Search

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Introduction: Preliminary screen of drugs and toxic compounds in various matrices is an important and challenging task performed by forensic and clinical laboratories. Traditional methods for preliminary screen include immunoassay (IA), gas chromatography-nitrogen/phosphorous detection (GC/NPD), mass spectrometry (MS), GC/MS, and liquid chromatography-diode array detection (LC/DAD). Recent advances in the LC-MS/MS technology have provided an opportunity for the development of more specific approaches to achieve the "screen" and "confirmation" goals in a single analytical step.

Objective: The objectives of this study are: (a) the establishment of chromatographic and mass spectrometric database including 1000 plus toxic compounds; and (b) the development of an effective UHPLC-QTOF/MS (ultra high performance liquid chromatography/quadrupole time-of-flight mass spectrometry) protocol for general unknown screen of these compounds for application in forensic and clinical laboratories.

Method: Liquid-liquid extraction procedure — using Toxi-tubes® A protocol — was coupled to an Agilent 6540 Q-TOF instrument equipped with a Jet Stream interface in combination with an Agilent 1290 Infinity LC instrument. Separation was achieved within 15 minutes, at a 0.31 mL/min flow rate, by gradient chromatography on Agilent Zorbax SB-Aq (2.1 x 100 mm, 1.8 µm) analytical column operated at 50 °C. Mobile phase consisted of solvent mixture composed of methanol and water containing 0.1% formic acid. Ions were generated in positive electrospray ionization mode. Samples were detected at 2 GHz single MS mode, m/z range 100–1000 with a scan rate of 2.0 spectra/sec. Data were acquired and processed with MassHunter B.05.00 software. An in-house database, comprising more than 1000 drugs and metabolites, was established using data resulting from the analysis of samples prepared from certified standards or other documented reference materials. The "Find-by-Formula" algorithm was used for data extraction. Matching tolerance parameters were: ±5-ppm mass accuracy; ±0.20-min retention time deviation; and ≥10000-count peak height. These matching parameters and isotope pattern were used to derive identification scores. Established protocol was used for the analysis of postmortem blood specimens for effectiveness assessments.

Results: Current database includes 1043 toxic compounds. The established method was applied to the analysis of 100 postmortem blood samples. The numbers of drug detected, by UHPLC-QTOF/MS, LC-IT/MS (LC/ion trap mass spectrometry) and GC/MS methods were 654, 351, and 130, respectively. The established method was found highly effective when applied to the analyses of postmortem specimens.

Conclusion: The over-all protocol provides a rapid, sensitive approach to isolate, screen, and confirm a broad spectrum of toxic compounds. No significant interference was found at the retention time expected of the targeted compounds. Preliminary data derived from the analysis of postmortem blood specimens are promising, significantly more effective than the RRLC-IT/MS and GC/MS approaches. More specific parameters, such as specificity and accuracy, of this method are currently under evaluation.

Keywords: UHPLC-QTOF/MS, General Unknown Screening, Postmortem Samples

P50

Rapid UPLC-MS^E/TOF Method for Simultaneous Quantitation of Cocaine and Its Metabolites Using Direct Extracts of Pulverized Human Hair

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Introduction: There is expanding use of hair testing for determination of drug exposure both for clinical and forensic purposes including verifying drug use history, investigating drug use in child custody cases, monitoring abstinence during parole, and documenting *in utero* exposure. Liquid chromatography-high resolution mass spectrometry technology (UPLC-MS^E/TOF) is validated for use in rapid detection and quantitation of cocaine and its metabolites using direct extracts of pulverized hair.

Objective: The objective of this study was to develop and validate a rapid, sensitive and specific assay for the analysis of cocaine, benzoylecgonine, cocaethylene and norcocaine in hair using UPLC-MS^E/TOF.

Method: After decontamination with dichloromethane and addition of analyte-specific deuterated internal standards, 20 mg of hair calibrators, controls and unknowns were pulverized for four minutes with metal beads using a Minilys homogenizer. The extent of mechanical disruption in pulverized hair was examined under electron microscopy. Analytes were extracted from pulverized hair by addition of acidified methanol (3.3% formic acid in methanol) and sonicated for 15 min. Following filtration with fritted pipette tips, the acidified methanol extracts were dried, reconstituted and analyzed directly by UPLC-MS^E/TOF using electrospray ionization in positive ion mode. Data was analyzed using TargetLynx software.

Results: Scanning electron microscopy revealed mechanical disruption of hair cuticles with exposure of the underlying cortical and medullary regions, allowing for rapid drug and metabolite extraction with only a 15 min sonication step. Linearity for all analytes was demonstrated by regression analysis over the range of 50 – 4000 pg/mg with correlation coefficients > 0.997. Limits of detection (LOD) and limits of quantitation (LOQ) were 25 pg/mg and 50 pg/mg, respectively, for all substances. Analytical performance parameters (*n = 10*) are summarized in the following table:

Total Precision CV (% Bias)	**Cocaine**	**Benzoylecgonine**	**Cocaethylene**	**Norcocaine**
QC1 (50 pg/mg)	19.5 (-8.7)	18.3 (-3.9)	18.4 (-4.0)	17.9 (-12.8)
QC2 (200 pg/mg)	9.4 (14.0)	11.5 (16.8)	10.2 (14.7)	9.1 (16.3)
QC3 (700 pg/mg)	3.5 (9.3)	5.4 (10.4)	3.7 (8.1)	5.6 (13.7)
QC4 (3000 pg/mg)	3.6 (0.2)	3.9 (0.2)	4.1 (0.2)	5.4 (4.1)
% Extraction Recovery at 100pg/mg (1000 pg/mg)	98 (84)	102 (85)	94 (81)	97 (81)
% Ionization suppression/Enhancement at 100pg/mg (1000 pg/mg)	-18.2 (-4.3)	-17.9 (-1.9)	-17.0 (-2.9)	-24.2 (-2.2)
% Process Efficiency at 100 pg/mg (1000 pg/mg)	80 (75)	82 (80)	79 (74)	74 (75)

Assay performance was further verified in cocaine users' hair with analysis of proficiency testing samples resulting in satisfactory Z-scores within $\pm$ 1.0.

Conclusion: Acceptable analytical performance has been verified for quantitation of cocaine and its metabolites in human hair by rapid extraction and UPLC-MS^E/TOF analysis. The method is applicable to a wide range of clinical and forensic toxicology uses and the methodology principles may also be adapted to the analysis of other drugs in human hair.

Keywords: Rapid UPLC-MS^E/TOF, Extracts of Pulverized Hair

P51

Validation of a Rapid and Robust Filtration Method for Determination of Total Opiates in Urine by Tandem Mass Spectroscopy

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Introduction: Opiates are natural or synthetic drugs that are used primarily for pain relief and often produce physical and psychological dependence. Opiates are excreted from the kidney in both free and conjugated forms. Appropriate clinical interpretation requires the total urine concentration, which is the sum of the unconjugated and conjugated forms of the opiates. Enzyme hydrolysis liberates the total drug concentration, and when combined with traditional dilute and shoot sample preparation can result in increased ion suppression and instrument maintenance. Therefore, aqueous filtration devices for sample preparation have begun to emerge as a means of reducing these concerns. Reported here is a rapid and robust liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the detection of codeine, morphine, hydrocodone, hydromorphone, oxycodone, and oxymorphone using an aqueous filtration device capable of automation.

Objective: The principle aim of this study was to develop a robust LC-MS/MS method to measure total opiate concentrations in urine that would be amendable to automation, have minimal cost, less ion suppression/ enhancement, and provide improved turn-around time.

Method: Urine (200 uL) was hydrolyzed, diluted, and filtered through a Clean Screen® FASt plate (UCT). Analytes were separated by a Restek Ultra Biphenyl column and analyzed on an ABSciex 4000 LC-MS/MS in positive ion mode with electrospray ionization, monitoring three product ions for each analyte. Deuterium labeled analogues of each analyte were used as internal standards. Total analysis time was 2.5 minutes per samples using a multiplexing system.

Results: The method was validated from 100-10,000 ng/mL with a limit of detection of 10 ng/mL for all analytes. The average linear regression (n=10) demonstrated the following: slope=1.0007; r^2=0.9997; and intercept=-5.312 for all analytes. Intra- (n=20) and inter-day (n=10) imprecision CV's were <4.8% and <5.8%, respectively, for all analytes across the analytical range. The recovery for all analytes was >83%. Accuracy was assessed by comparison with an outside laboratory, and showed a mean difference of <10% for morphine, oxycodone, and hydrocodone. Codeine, oxymorphone, and hydromorphone, which are known to be more difficult to hydrolyze enzymatically, yielded a mean difference of -14%, -22%, and -23%, respectively. No analytical interferences were found amongst 65 commonly prescribed and abused drugs, including tramadol, tapentadol, 6-monoacetylmorphine, 3-monoacetylmorphine, dihydrocodeine, and norhydrocodone. In addition, minimal to no ion suppression or enhancement due to the matrix effect was observed.

Conclusion: A rapid method amendable to automation for the quantitation of total opiates was validated and offered significant improvements over basic dilute and shoot LC-MS/MS methods. This method utilized an aqueous filter plate which was employed after sample dilution, and virtually eliminated any ion suppression or enhancement. The differences in accuracy observed between methods are attributed to non-standardized hydrolysis conditions between laboratories. Results from samples that did not contain conjugated analytes matched within ±10%, confirming that any variation seen was due to differences in the hydrolysis procedures.

Overall, this method provides cleaner samples, increased robustness and is amendable to automation.

Keywords: Opiates, LC-MS/MS, Automation

P52
A Quadrupole GC-MS with Dual-Role Toxicology Capability

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Introduction: Gas chromatography-mass spectrometry (GC/MS) is the established gold standard method of clinical and forensic toxicology analysis. Toxicology laboratories typically use either ion trap or quadrupole GC/MS instruments for drug analysis. The trapping characteristics of ion traps promote high sensitivity, and are therefore preferred for qualitative analyses such as screening for drugs. Alternately, quadrupole instruments are more often applied in quantitative analyses due to superior linear response.

Recently, we observed unexpectedly high sensitivity with a new quadrupole GC/MS (ISQ, Thermo Scientific Inc.). Following successful validation of quantitative THC and cocaine metabolite methods, we conducted a study to compare the sensitivity of the ISQ quadrupole instrument to that of the Thermo Scientific ITQ ion trap instrument in our laboratory.

Objective: The objectives of this study were to evaluate the sensitivity of the Thermo Scientific ISQ quadrupole GC-MS, and determine its effectiveness for unknown toxicology screening via comparison with the Thermo Scientific ITQ ion trap platform.

Method: We began by cleaning each instrument and installing identical new columns (Restek Rtx®-5MS). Subsequently, 100 urine samples, commercial controls, and proficiency test specimens were analyzed as approved by the Institutional Review Board at the University of Mississippi Medical Center, Jackson, MS. Each sample was extracted via solid phase extraction (Bond Elut Certify), and injected in random fashion onto the Thermo ITQ and Thermo ISQ instruments for a side-by-side comparison. All spectral analyses were performed manually by an experienced medical technologist using predetermined purity and fit criteria for identification.

Results: Equal numbers of identical drugs were detected by each instrument in the commercial controls and proficiency test samples analyzed. A higher number of drugs in patient samples was detected with the ISQ quadrupole than with the ITQ ion trap. While sample agreement was very high, more drugs were detected with the ISQ quadrupole instrument in all incidents of non-concordance. This likely resulted from a combination of higher sensitivity of the ISQ, and higher baseline noise on the ITQ which was noted in select patient samples analyzed near the end of the study. As a result of this study, the Thermo ISQ GC/MS was validated and has been employed in our laboratory for both qualitative toxicology screening and quantitative drug confirmations.

Conclusion: These data indicate that the ISQ quadrupole GC/MS exhibits comparable sensitivity to that of the Thermo ITQ ion trap GC/MS. The sensitive ISQ quadrupole system offers laboratories the opportunity to use a single instrument for quantitative and qualitative analyses. High volume labs could be supported by multiple ISQ instruments, which would confer backup capability. Additionally, use of a single instrument type could reduce job complexity by eliminating the requirement to learn multiple types of software on different instruments.

Keywords: Forensic Toxicology, GC/MS, Ion Trap, Quadrupole

P53

Comprehensive Screening and Quantitation of Designer Drugs by LC-QQQ-MS/MS Analysis

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Introduction: In recent years there has been a surge in the emergence of novel designer drugs due to the clandestine synthesis of these drugs by amateur chemists. To date, the major classes of designer drugs include phenethylamines, cathinones, tryptamines, piperazines, and synthetic cannabinoids, all of which evoke psychoactive effects similar to classic drugs of abuse. The introduction of these entities into the illicit drug market has resulted in larger profits for manufacturers and distributors, and an ever-increasing threat to public health. In 2012, the U.S. Congress passed legislation to schedule 26 designer drugs, and the Pharmaceutical Administration and Regulations of Japan also banned 13 designer drugs. The prevalence of rapidly evolving chemical structures of designer drugs is a direct consequence of strategic moves on the part of illicit drug manufacturers to circumvent current regulations of drugs of abuse ultimately making their analysis difficult. Thus there is a need for a comprehensive analytical method which can effectively screen, confirm, and quantify all major classes of designer drugs.

Objective: To develop a comprehensive designer drug screening library by providing specific triggered multiple reaction monitoring (tMRM) transitions, chromatography, and informatics tools to assess the presence of these drugs in relevant toxicological matrices. The proposed library generates a score match value when comparing against unknown samples which will enhance the ability to accurately identify different classes of designer drugs under scrutiny.

Method: An Agilent 1290 Infinity LC coupled to an Agilent 6460 triple quadrupole MS/MS (LC-QQQ-MS/MS) instrument was used to develop a rapid, sensitive, selective, and comprehensive analytical method which possesses the capability to effectively screen, confirm, and quantify several hundred designer drugs of all major classes. Within this method a dynamic/triggered MRM method was incorporated, for which fragmentation pattern data consisting of up to 10 ion transitions for each analyte were obtained. Chromatographic separation of the target analytes was achieved by using a Zorbax Eclipse Plus C_{18} column, 2.1 x 100 mm, 1.8 µm, maintained at 40°C and eluted with a gradient of A) aqueous 5 mM ammonium formate and B) methanol, both containing 0.1% formic acid. An MRM method combining the transition and retention time data of each designer drug compound was used to analyze a super mix consisting of 275 designer drugs.

Results: Separation of all DEA and Japan regulated drug entities in less than 16 min was achieved. Fragmentation pattern data consisting of up to 10 transitions per analyte were acquired for approximately 275 designer drugs. Acceptable linearity (r^2>0.99) and detection limits in the low ppb range were obtained for the majority of the investigated analytes. The transitions and retention times obtained were incorporated into a customized database to allow identification via a score match.

Conclusion: This method is effective in producing characteristic MS/MS spectra and chromatography specific to several hundred designer drugs. Coupled with the MRM library, the method is adequate to screen, confirm, and quantify designer drugs from all classes, thereby making it applicable for use in forensic analysis of biological matrices. Current work is examining application of the method to authentic casework specimens.

Keywords: Designer Drugs, LC-MS/MS, Cathinone Derivatives, Synthetic Cannabinoids, Triggered MRM

P54

Determination of Synthetic Cathinones in Oral Fluid by Liquid Chromatography-Tandem Mass Spectrometry

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Introduction: Synthetic cathinones have recently emerged as a substitute for common drugs of abuse. These drugs can elicit powerful adverse effects such as delusions, hallucinations, and potentially dangerous behavior. Considering the increased use of synthetic cathinones and their adverse effects, it is essential to develop analytical techniques that identify and quantitate them accurately.

Objective: To develop a method to analyze ten synthetic cathinones in oral fluid, implementing a combined approach of solid phase extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Method: The method development and validation were performed in oral fluid matrix. During the solid phase extraction method, the drugs were eluted with a small amount of elution solvent, so that the sample could directly be injected into the LC-MS/MS instrument. Tandem mass spectrometry was performed using the positive electrospray ionization method with multiple reaction monitoring mode. Two ion transitions were monitored for each analyte.

Results: The developed method was able to quantitatively identify MDPV, mephedrone, flephedrone, methedrone, ethylone, butylone, methcathinone, methylone, PVP, and pyrovalerone. The chromatographic gradient run time was 3 min. The linear dynamic range was 1-500 ng/ml with a lowest limit of quantitation (LLOQ) of 1 ng/ml for each analyte. Inter-day accuracy and intra-day accuracy were within the accepted limit of ≤15% (≤20% at LLOQ) and inter-day precision and intra-day precision were within the accepted limit of ≤15% (≤20% at LLOQ) for all the analytes.

Matrix effect and the recovery were calculated for two different concentrations (50 ng/mL and 500 ng/mL). The developed extraction procedure was able to remove matrix interferences from the oral fluid samples and, as a result, the LC-MS/MS analysis suffered from relatively low ion enhancement or ion suppression effects for all the target analytes. The method showed satisfactory recoveries for most of the analytes. MDPV, pyrovalerone and PVP exhibited relatively lower recoveries; however, they were quantitatively identified in low concentrations because of the sensitivity of the LC-MS/MS method: LLOQ for MDPV, pyrovalerone and PVP was 1 ng/mL.

Human subject samples were analyzed using the developed method to demonstrate the applicability of the method. Prior to initiation of the research study, all human subject samples were de-identified and any demographic data or other means of patient identification were permanently deleted from the sample records. From December 2012 to January 2013, the laboratory received 1915 oral fluid samples with requests to analyze synthetic cathinones. Five samples were found to be positive for MDPV and PVP. Concentration of human subject samples ranged from 20.0 - 837.7 ng/mL for MDPV and 81.9 - 935.0 ng/mL for PVP.

Conclusion: Our work summarizes the validation of an integrated method of solid phase extraction and LC-MS/MS that can quantitatively identify ten synthetic cathinones in oral fluid. This is a rapid and robust method that can be used in high-throughput toxicological screening applications.

Keywords: Synthetic Cathinones, Bath Salts, LC-MS/MS, Solid Phase Extraction

P55
Quantitative Analysis of XLR-11 and UR-144 in Oral Fluid

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Introduction: Availability and consumption of synthetic cannabinoids have risen recently in the US and Europe. These drugs have adverse effects, including acute psychosis and bizarre behavior. In 2011, US DEA added five of the synthetic cannabinoids to Schedule 1 of the Controlled Substances Act. As synthetic cannabinoid strains are added to the Schedule 1 list, new strains are being introduced to the market. UR-144 and XLR-11 are two of the most recent additions to the synthetic cannabinoid drug class. The continuous addition of new synthetic cannabinoids to the market makes it extremely difficult to control these new synthetic drugs. Currently available screening and confirmation methods do not detect any of the new compounds; therefore, it is important to develop new techniques to identify new synthetic drugs.

Objective: To develop a rapid and sensitive LC-MS/MS method for quantitative measurement of UR-144, XLR-11 and their metabolites 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 Oasis µ-elution MCX cartridge. Only 400 µL of the collected sample was used for the SPE.

Chromatographic separation was performed on an Acquity UHPLC system (Waters) equipped with an Acquity BEH (C-18, 100 x 2.1 mm, 1.7 µm) analytical column (Waters). Run time was 3 minutes. Electrospray ionization mass spectrometry was performed on a TQD instrument (Waters). Analysis was performed in positive ionization (ESI+) and multiple reaction monitoring mode (MRM). Two transitions, quantifier and qualifier, were used to identify the target analytes. MRM transitions for the parent drugs were as follows: 330.4>125.1 m/z and 330.4>232.1 m/z for XLR-11 and 312.4 >125.1 m/z and 312.4>125.1 m/z for UR-144.

Results: The developed method was validated according to internationally accepted guidelines. The linear dynamic range was 5-100 ng/ml with a lowest limit of quantitation (LLOQ) of 5.0 ng/ml for each analyte. Inter-day accuracy and intra-day accuracy were each within the accepted limit of ≤15% (≤20% at LLOQ) and inter-day precision and intra-day precision were each within the accepted limit of ≤15% (≤20% at LLOQ) for all the analytes. In addition, sample stability, selectivity, matrix effect and recovery were calculated.

Conclusion: Authentic samples (n=43) were tested to evaluate the applicability of the method. 14 samples were found to be positive for XLR-11 (5.0-86.8 ng/mL) and 1 sample was found to be positive for UR-144 (9.5 ng/mL).

Keywords: XLR-11, UR-144, LC-MS/MS, Oral Fluid, Synthetic Drugs

P56
Oral Fluid Instant Drug Testing: Evaluation of 13 Point-of-Collection Testing Devices

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Introduction: Oral fluid (OF) is becoming an increasingly popular matrix for testing drugs. There are many advantages to using OF as opposed to more traditional matrices like blood and urine, including: less invasive collection, easy observation, adulteration is difficult, parent drugs can be detected, and drug concentrations generally correlate with blood levels. As OF testing becomes more prevalent, the need for accurate and sensitive point-of-collection testing (POCT) devices will be vital to drug testing in driving under the influence of drugs (DUID) cases and therapeutic drug monitoring.

Objective: To compare the accuracy, sensitivity, and specificity of commercially available POCT OF devices. The devices evaluated in this study include: Drugwipe® (Securetec), Oratect® HM15 (Branan Medical Corp.), Stat Swab™ (Micro Distributing), Swab Cube™ (Premier Biotech), Oral Tox™ (Premier Biotech), Discover Cassette (American Screening Corp.), Oraline® IV SAT (American Screening Corp.), Oral Cube™ (W.H.P.M. Inc.), SalivaScan™ (Drugcheck), SalivaConfirm® (Confirm Biosciences), Oralstat® (ABMC), OrAlert™ (Innovacon), and iScreen® (Alere).

Method: Each device was evaluated at 5 different concentrations based on manufacturer cut-offs(x): Negative (n=5), 0.5x (n=10), 1.25x (n=10), 2x (n=10), and 10x (n=10; for a total of 45 samples). Oral fluid samples were prepared in negative OF calibrator (Orasure Technologies), spiked with appropriate analyte for each device, and confirmed by LC/MS/MS. Analytes included Amphetamine, Methamphetamine, Cocaine, Phencyclidine (PCP), Benzodiazepines, Barbituates, Buprenorphine, Opiates, Oxycodone, Propoxyphene, Methadone, and Δ9-tetrahydrocannabinol (Δ9-THC). Collection pads were incubated in 3 mL of spiked OF and manufacturer instructions followed. Results were reported as true positive (TP), true negative (TN), false positive (FP), or false negative (FN).

Results: Every POTC device performed well for opiates (> 77% accuracy, >66% sensitivity) with several devices detecting opiates below their cut-off. Amphetamine and methamphetamine accuracy ranged from 55.6-100% and 55.6-100%, respectively. Except for 4 devices, cocaine accuracy was greater than 50% (7 devices above 77%). Most devices that detected PCP had accuracies of greater than 60%. Many FP and FN were observed for Δ9-THC and over half had sensitivities below 50%. There was a wide range of variability in the ability of the POCT devices to detect other drugs, and many had poor sensitivity near their cut-off concentrations. The only device to have 100% sensitivity for all drugs tested was Drugwipe®.

Conclusion: Based on suggested SAMHSA cut-offs, there is room for improvement in the functionality of POCT devices for OF. The sensitivity for Δ9-THC remains insufficient due to either high cut-off concentrations (above SAMHSA suggested 4ng/mL) or inability to detect the parent compound. Oral fluid POCT devices work at high concentrations for most drugs and may be suitable in cases of drug abuse. Although collection pads are able to absorb ample OF in a laboratory setting, issues remain as to whether the devices can collect enough OF to perform the test in a real collection environment, where OF collection from donors may vary due to varying OF secretion, medical conditions or drug use. For these reasons it may be difficult to obtain enough sample for to perform POCT and laboratory conformational analysis.

Keywords: Oral Fluid Instant Drug Test, Point of Collection Testing (POCT)

P57

Investigation Into the Stability of Ethanol in Blood Samples Collected for Suspected Driving Under the Influences (DUI) Cases

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Introduction: Blood Alcohol Concentration (BAC) is used to measure alcohol intoxication for legal or medical purposes. In a Toxicology Laboratory, whole blood ethanol test results are one of the most important measurements for suspected DUI drivers. Due to legislations such as *Melendez-Diaz* whereby original ethanol analysts are required to testify, there is often a need to re-analyze blood samples due to analyst availability. Questions may arise about ethanol depreciation in storage. Variation can be due to factors such as; ethanol and preservative concentration, collection tube type, sample volume, frequency of opening, and time and storage conditions.

Objective: The investigation objective was to compare BAC results of samples that have been retested at a different time points to determinate the stability of blood ethanol. In addition, 20 samples were selected from different storage ages to identify if ethanol production occurs when blood samples are in long-term storage.

Method: Fifty whole blood samples submitted to the laboratory as suspected DUI cases were selected for this study. Most tested samples (n=48) were stored refrigerated (2-8°C) between 0.5-8 yr; some samples (n=2) >6 yrs old were stored at room temperature prior to reanalysis. Samples were collected and stored in grey stopper vacutainers (GSV) containing 100mg sodium fluoride and 20mg of potassium oxalate. Ethanol quantitation was determined by Head Space Gas Chromatography (HS/GS) dual column analysis. This method utilizes 1N-propanol as an internal standard with a 6 point calibration (0.025 – 0.3 g/100mL) demonstrating linearity of R^2 of ≥0.999, alongside negative, low and high controls.

Results: Samples negative for ethanol (n=13), when repeated were also negative for ethanol, regardless of storage condition or length. Four samples repeated within one month after refrigeration showed no variation (within analytical range of the method ± 5%) in ethanol concentration. The comparative data for the remaining samples (n=33) demonstrates some variability; however, overall the data shows a normal distribution. Mean and median losses were 6% and 5% decrease/year respectively (range from 2-12% decrease/year). Three samples analyzed fell outside this range showing percent losses of 22, 23 and 38%/year; these samples were excluded from mean and median calculations.

Conclusion: Overall this study demonstrates that blood collected in GSV and stored for a period of ≤8 yrs demonstrates no increase in ethanol concentration, regardless of storage temperature. This stability study identified a range of 2-12% decrease/yr when blood is stored under refrigerated conditions ≤8 yrs; this information should be considered when interpreting repeat analyses for ethanol. The sample that demonstrated significant ethanol deprecation (38%/year loss) contained limited blood volume (~2mL) in Tube A. When Tube B was opened and analyzed, the values then fell within the study range (5%/yr loss). To hypothesize the large headspace above the sample in Tube A may have contributed to the larger decrease. Two samples that demonstrated a larger than average depreciation were multi-drug positive, resulting in the tube being re-opened many times for qualitative and quantitative drug analysis before ethanol was re-tested, possibly allowing for ethanol evaporation.

Keywords: Stability, Blood Sample, Ethanol, DUI

P58

Optimization of a Method for the Quantification of Underivatized Amphetamines in Whole Blood Using Headspace Solid-Phase Microextraction and Fast GC-MS/MS

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Introduction: Methamphetamine (MET) and amphetamine (AMP) are powerful stimulants that are often abused and could lead to serious addiction and death. The development of a rapid and robust method for analyzing these drugs in postmortem whole blood would benefit forensic toxicology laboratories.

Objective: The objective was to develop a fast and specific method for analyzing amphetamines from whole blood without derivatization.

Method: Amphetamine-d_7 (AMP-d_7) and methamphetamine-d_8 (MET-d_8) was added to 0.1 g of whole blood and 100 µl of 5M NaOH in a 20 ml glass vial and immediately capped. The vial was heated at 220°C to volatilize the analytes. The SPME fiber was exposed to the heated headspace and after 7 minutes retracted and inserted into the injector port of the GC for desorption and analysis. The samples were analyzed by gas chromatography-tandem mass spectroscopy (GC-MS/MS) on a Shimadzu GCMS-TQ8030 equipped with a tandem capillary column. The tandem column was composed of a BPX5 (1.3m x 0.25mm I.D., df=0.5µm) coupled with a BPX5 (4m x 0.15mm I.D., df=0.25) (SGE, Australia). The injection port temperature was 280°C and the interface temperature was 230°C. The initial oven temperature was 80°C, held for 0.35min, increased at 50°C/min to 340°C, and held again for 0.35min. Chemical ionization (CI) using isobutane as the reagent gas was employed, with the CI voltage set at 150 eV. Multiple Reaction Monitoring (MRM) was utilized to identify and quantify the target analytes. The MRM transitions for AMP-d_7, AMP, MET-d_8, and MET were 143>126 (CE 6V), 136>119 (6V), 158>124 (9V), and 150>119 (6V), respectively. The retention times for AMP-d_7, AMP, MET-d_8, and MET were 0.80, 0.81, 1.06, and 1.08 minutes, respectively.

Results: This method was effective in detecting and quantifying the above amphetamines quickly and without derivatization. The retention time was under 2 minutes for all analytes. Quantitation curves were comprised of concentrations at 0.1, 0.5, 1.0, 5.0, and 10 µg/ml. Correlation coefficients of both curves were 0.995 or better. Utilizing the MRM method allowed for detection of the analytes down to 10 ng/ml, however quantitation was performed from 0.1µg/ml, similar to conventional GC methods. Reproducibility was confirmed using quality control (QC) samples spiked at 0.5 and 5.0µg/ml and monitored over three consecutive days. The QC samples never differed more than 7% from the expected value. Methamphetamine concentrations of actual case samples quantified using the new method were found to be within 20% of the previous results analyzed using a conventional GC-MS method. Total run time for one sample from sampling to completion was under 20 minutes.

Conclusion: The extraction of methamphetamine and amphetamine was accomplished while avoiding the time and cost of conventional extraction methods like liquid-liquid and solid-phase extraction. The employment of fast GC-MS/MS and the MRM mode in a triple quadrupole system allowed for highly specific and sensitive analysis of these drugs quickly and without derivatization. This method could be easily employed by laboratories desiring a quick and robust way to screen and quantify amphetamines in their casework.

Keywords: Methamphetamines, Headspace, Solid-Phase Microextraction, Fast GC-MS/MS

P59

Analysis of Pooled, Anonymous Urine from a Nightclub Using UPLC®-TOF-MS^E

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Introduction: In a study designed to assess the feasibility of using pooled urine to confirm what drugs are currently being used in the night-time economy, a series of pooled samples were collected at a London nightclub.

Objective: The samples were analysed using a variety of techniques - this paper presents the results of screening using UPLC-TOF-MS^E.

Method: Pooled urine samples were collected from an adapted portable urinal; use of the urinal was anonymous and voluntary. Four samples were collected at different time points throughout the evening. Samples were analysed using UPLC in combination with TOF-MS^E. Chromatographic separation was achieved using a Waters UPLC® I-Class system fitted with a HSS C_{18} column maintained at 50^{o}C and eluted with a mixture of ammonium formate (pH3) and acetonitrile containing 0.1% formic acid. The total chromatographic run time was 15 min. Data were collected using a Xevo G2S-QTOF in MS^E mode; this involves the rapid alternation between two functions: the first, acquired at low energy, provides accurate mass of the precursor ion; the second, at elevated energy (ramp 10-40eV), provides accurate mass of the fragment ions for additional confirmatory purposes. Data were processed using the UNIFI™ Toxicology Screening Solution (Waters) and compared to a comprehensive database, prepared under the same conditions, containing > 1000 drugs and metabolites.

Results: Substance identification was based on retention time and an accurate mass 'fingerprint' for each analyte, the latter comprising accurate mass of the precursor ion and at least one additional specific fragment ion. Typically, for each substance in the database, a range of one to four additional fragments ions were specified for each substance. A number of classical recreational drugs were identified including: amphetamine, methamphetamine, MDMA, ketamine, and morphine. The screen also identified a number of novel psychoactive substances including 4-methylmethcathinone (mephedrone), 4-methylethcathinone and TFMPP (3-trifluoromethylphenylpiperazine). Several potential drug adulterants were also detected as well as prescription/over-the-counter medications.

Conclusion: The study demonstrated the utility of using pooled samples to confirm recreational drug use. Analysis by TOF-MS^E permitted detection of drugs and their metabolites in diluted urine samples. The technique is particularly suited to screening for novel drugs in the absence of standard reference material.

Keywords: Recreational Drugs, Mephedrone, Pooled Urine, UPLC-TOF-MS^E

P60

High Performance Liquid Chromatography Tandem Mass Spectrometry Method for the Determination 2CC-NBOMe and 25I-NBOMe in Human Serum and Accompanying Overdose Case Reports

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Introduction: A high pressure liquid chromatography with electrospray ionization triple quadrupole mass spectrometry (HPLC/MS/MS) method was developed in response to an outbreak of N-benzyl-phenethylamines derivative abuse and non-fatal overdose cases in our state during the early part of 2012. 4-chloro-2,5-dimethoxyphenethyl)-N-[(2-methoxyphenyl) methyl] ethanamine) (2CC-NBOMe) and 2-(4-iodo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl) methyl] ethanamine) (25I-NBOMe) are N-benzyl phenethylamines derivatives whose synthesis was first reported in the scientific literature in 2011. In vitro binding studies have demonstrated that these compounds are potent serotonin 5-HT2A receptor agonists. The 5-HT2A receptor has been closely linked to complex behaviors including working memory, cognitive processes and affective disorders such as schizophrenia. These receptors are believed to mediate the primary effects of hallucinogenic drugs. Little to no pharmacokinetic or pharmacological data concerning man or whole animals is presently available in the professional literature.

Objective: Identify and quantify 2CC-NBOMe and 25I-NBOMe in serum specimens from intoxicated patients.

Method: A HPLC/MS/MS method for the detection and quantification of 2CC-NBOMe and 25I-NBOMe in serum is presented. The assay applies 2-(2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine (25H-NBOMe) as the internal standard (ISTD) using solid phase extraction (SPE) with ZSDAU020 Clean Screen Columns. The separation of the drugs was performed using a Luna 3u C8(2)100A 100x200 mm, 3 micron column . The mobile phase contained water/acetonitrile (20:80 v/v) with 1% formic acid delivered at a flow rate of 0.3 mL/min. The mobile phase consisted of A: Water with 10mmol ammonium acetate and 0.1% formic acid and B: Methanol.

The following gradient was used: 0.00-1.10 min at 20% B, a linear gradient to 40% B until the 6.00 min, hold for 2 min, then return to 20% B at 8.00 min. The retention times were: 2C-2, 4.4 min; 25I-NBOMe 4.8 min and 25H-NBOMe, 3.8 min. The following transition ions (m/z) were monitored in multiple reaction monitoring (MRM) mode: 2C-C; 336>121 and 336>91, 25I-NBOMe; 428>121 and 428>91 and 25H-NBOMe; 302>121 and 302>91.

Results: Assay performance was evaluated using a seven point calibration curves analyzed in duplicate (30-2000pg/mL) and a set of four quality control specimens. Accuracy and bias of the assay was determined to be within ± 15% of the target value for each analyte at each quality control value. Intra-day and inter-day precision samples were determined not to exceed a 15% CV and 20%, respectively, for the LOQ demonstrating acceptable precision for samples run in one day as well as for independent runs over three days. The method was also evaluated for absolute recovery, ion suppression, carryover, specificity and stability. The method was used to identify and quantify 25I-NBOMe in serum from two severely intoxicated patients at concentrations of 250 pg/mL and 2,780 pg/mL whose case histories will be presented.

Conclusion: The method has been found acceptable to detect and quantify 2CC-NBOMe and 25I-NBOMe in patient serum specimens.

Keywords: 25I-NBOMe, 2CC-NBOMe, HPLC/MS/MS, Designer Drugs

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P61

Development and Validation of a New Homogeneous Immunoassay for the Detection of UR-144 Metabolites in Urine

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Introduction: Synthetic cannabinoids are consistently being synthesized worldwide with the intention of evading detection in biological matrices.

Objective: The objective of this project was to develop and validate a new high throughput homogeneous enzyme immunoassay (HEIA) for the rapid detection of the urinary metabolites of the latest synthetic cannabinoids such as UR-144 and XLR-11. Despite the fact that UR-144, XLR-11, JWH-018, and AM2201 belong to a drug class that share a core indole-ring moiety, it is challenging to develop an immunoassay to cross react with all of them due to the significant difference in structures of the substituted groups. The current commercially available homogeneous immunoassay targeted at JWH- metabolites has very low cross reactivity for UR-144 and XLR-11, thus it was necessary to develop a new screening method to detect UR-144, XLR-11 and their metabolites in urine.

Method: An anti-UR-144 polyclonal-based homogeneous immunoassay was developed and validated with authentic urine specimens previously confirmed by LC-MS/MS. The assay was designed to detect UR-144 pentanoic acid, a major metabolite of UR-144.

Results: The reportable range of the assay was 5 to 40ng/mL while the cutoff concentration of UR-144 pentanoic acid was set at 10ng/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 65 urine samples previously analyzed by LC-MS/MS. The sensitivity, specificity and accuracy of the assay were found to be 96%, 100% and 98%, respectively.

		Confirmation (1ng/mL)	
		Negative	**Positive**
HEIA (10ng/mL)	**Negative**	40	1
	Positive	0	24

Conclusion: A high throughput homogeneous enzyme immunoassay has been developed for the detection of UR-144 and XLR-11 metabolites in human urine. When applied to authentic specimens the assay correlated well with LC-MS/MS results.

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Keywords: UR-144, XLR-11, Synthetic Cannabinoids, Spice, K2, Immunoassay

P62

Functional Self-Assembly Thin Film For Highly Sensitive Detection of Codeine Using Surface Plasmon Resonance

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Introduction: Drug-related criminal cases especially involving codeine-abuse has increased in China. To date, detection methods for codeine and its analog morphine have traditionally been based on gas chromatography (GC), high performance liquid chromatography (HPLC), radio immunoassay in the lab. Because of increasing drug-related cases, it is necessary to find a screening approach that could improve the simplicity, efficiency and sensitivity in a quick drug test. Sensory technology based on molecular imprinting polymers (MIP) has attracted considerable interest due to its high specificity for binding analytes through covalent or non-covalent interactions between the functional monomers and the target molecules. In the past years, the molecular imprinting method has been applied to the detection of a wide range of analytes including biological molecules, environmental pollutants or explosive compounds. Thus, the use of molecularly imprinted polymers (MIP), which fulfill both economic and stability criteria, is considered a more reliable and robust strategy for drug specific binding.

Objective: We combine a codeine sensing membrane with a sensitive transducer based on surface plasmon resonance (SPR) which can be applied into portable instruments in the future for an on-the-scene determination in order to significantly improve the efficiency and availability of routine drug analysis in actual forensic cases. To the best of our knowledge, this is the first example of codeine detection using SPR-based sensor with MIP. This approach features a rapid response, good selectivity, label-free detection and most notably, a very low limit of quantification (LOQ).

Method: The SPR chip was prepared by polymerizing the functional monomers directly on the gold-coated silylated glass. For the polymer, we selected methacrylic acid (MAA) as the functional monomers; ethylene glycol dimethacrylate (EGDMA) as the cross-linker, and azodiisobutyronitrile (AIBN) as the initiator. Codeine MIPs were prepared by carefully heating 7.0 mg of codeine free base and 8.6 mg of MAA until a homogeneous phase formed. Then, 0.04 g of EGDMA, 2 mL of acetonitrile, and 3 mg of AIBN were successively added and the mixture was purged with nitrogen and polymerized on gold (50 nm) SPR chips under nitrogen at 60 for 4h. Surface morphology of the MIP film was observed by scanning electron microscopy, which showed even and closely packing. The polymer films were cooled at ambient temperature and the chips were installed in the SPR machine. After that, the films were washed with acetonitrile and 4% acetic acid until a stable baseline was reached. After elution, the codeine solutions (acetonitrile) with different concentration (10^{-9} mol/L, 10^{-8} mol/L, 10^{-7} mol/L, 10^{-6} mol/L) were injected. In order to test the selectivity of the sensor, a solution of morphine analogue (10^{-4} mol/L, more than 105 times to the LOQ of codeine) was also injected.

Results: Upon adding increasing amounts of MO into the injected solution, the angle shift of the SPR response increased gradually. Notably, there exists an excellent linear relationship between the angle shift ($\Delta\theta$) and the logarithm of the concentration of MO (log [MO]) in the range of 10^{-9} M to 10^{-6} M. The above approaches require small amounts of codeine (7mg) and the relative signal of surface plasmon resonance (SPR) response is proportional (R^2=0.99131) to the concentration of codeine acetonitrile solution in the range of 10^{-9} to10^{-6}M （3–30000 ng/mL） with a detection limit (S/N = 3) of 1.3 ng/mL. The method has a good reproduction, with RSD of the resonance angle lower than 1%.

To elucidate the specificity of the MIP film-coated sensor for codeine, a structurally related analog, morphine, was also tested with the same protocol. It was found that upon injection of a high concentration of morphine solution, the sensing membrane with codeine-cavities detects negligible resonance angle shift, unlike the significant shifts obtained for codeine at much lower concentration (10^{-9} M). This result should be attributed to the methyl ether group (-OCH3) substituting the phenol group (-OH) in the 3-position of codeine. Therefore, one can reasonably conclude that hydrogen bonding must play a crucial role in promoting the absorption of MO in our MIP-film. As a result, the MIP film-coated sensor can easily discriminate codeine from morphine at low concentration levels.

Conclusion: The method showed high specificity, and the relative signal of SPR response is proportional to the concentration of codeine. The sensor can be applied in the biological samples directly such as urine and blood, which is still in progress currently. The above approaches require small amounts of polymer and this makes sensor fabrication feasible even for expensive target molecules such as toxins. The method will significantly improve the efficiency and availability of routine drug screening in actual forensic cases.

Keywords: Codeine, Molecularly Imprinted Polymers (MIP), Surface Plasmon Resonance (SPR)

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Microanalysis of Carbon Monoxide in Decomposed Blood and Hepatic Tissues by Headspace Gas Chromatography and Mass Spectrometry

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Introduction: During the past 20 years, several methods for the determination of postmortem COHb levels have been published. These methods include UV and FTIR spectrophotometry, CO-oximetry, and Capillary Electrophoresis (CE). Although gas chromatographic techniques are more suitable for forensic materials, they are complicated, time-consuming and require larger samples, when compared with CO-oximetry and GC/MS. However, CO-oximeters are neither capable of detecting COHb less than 10% nor performing measurement in putrid blood. In China, most of postmortem samples collected in suspected CO poisoning cases are not suitable for analysis because spoiled, deceased being often discovered days or even months after death. In such situations, quantification of COHb concentrations in spoiled blood using existing techniques may prove very difficult for the toxicologist.

Objective: In this paper, head-space gas chromatography-mass spectrometry (HS/GC/MS) techniques are used in order to establish a systematic method for measurement of CO levels, and provide an experimental basis for investigation of potential CO poisoning cases.

Method: Fresh blood samples were stored at room temperature to be spoiled for liner equation experiments. In order to the detect COHb% in tissues, the experiment was performed to make male rabbits inhale CO in the exposure apparatus. Immediately after the death of rabbits, the blood and tissues were collected and analyzed for COHb% by means of HS/GC/MS. All the samples were preserved in different temperature, and was detected by after it had been preserved for 7 days, 14 days, and 45 days at room temperature 17℃, -4 ℃and -20℃. The percentage saturation of blood with CO was calculated by the ratio of the peak areas obtained from untreated blood (Ac) and CO saturated blood (As):Ac/As × 100% = COHb% .

When dealing with the putrid blood in CO poisoning cases, in order to saturate hemoglobin CO was bubbled though the same blood from the deceased. This excludes the effects of variable Hb concentration when dealing with putrid samples which maybe showing low or high Hb concentrations due to unequal setting of blood.

Results: Using a packed molecular sieve column, we were able to quantitate CO levels down to 0.01% in the air and COHb levels down to 0.2% in small blood (0.25ml) and liver (1g) samples. It was shown that the method has good reproducibility with RSD of the COHb <1%. There is an excellent linear relationship (R^2=0. 9995) between CO peak areas (Y) and COHb% (X) with an excellent reproducibility (RSD<5%) in putrid blood. COHb% in hepatic samples of forensic cases are determined and showed good results, which were stored at different temperatures (-20°C for 1-2 years, 0°C, and 18°C for two months).

Conclusion: HS/GC/MS methods may have significant advantages over other available techniques for COHb% analysis. According to our method, it was shown that the effect of preserved temperature on the %COHb determination was negligible. The %COHb levels in the putrefied blood samples showed good consistency with the %COHb levels determined in the fresh blood, which was confirmed in forensic cases. Therefore, this technique provides an accurate and reliable method for determining CO and COHb% levels and may prove useful for investigation of deaths potentially related to CO exposure.

Keywords: Carbon Monoxide Poisoning, COHb, Decomposed Blood, Headspace Gas Chromatography/Mass Spectrometry (HS-GC /MS)

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Detection and Quantitation of Synthetic Cathinone by ELISA, GC/MS (scan) and GC/MS Selective Ion Monitoring (SIM) in Urine Driving Under the Influence Cases (DUI)

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Introduction: "Bath Salts" are the street name for synthetic cathinones. This group of compounds has similar effects to amphetamines, methamphetamines and MDMA (methylenedioxymethamphetamine). The use of cathinones has rapidly increased in recent years due to the ease of purchasing these drugs at local gas stations and head shops. Although there is a list of at least 40 known "Bath Salts," methylone and mephedrone are the most commonly seen in toxicology analysis. In forensic science to identify a compound there is a need for two independent methods- described in this paper.

Objective: To develop and validate a comprehensive testing method for some of these synthetic cathinones by using ELISA, GC-MS full scan and GC-MS SIM (selective ion monitoring) and to correlate the use of these drugs with the physiological and psychological effects as indicated by the drug recognition expert (DRE) report.

Method: Nine forensic urine samples that were initially screened as presumptive positive by ELISA for methamphetamines were selected. Samples were then analyzed by a basic drug screen (BDS) method utilizing GCMS using full scan mass spectra which identified methylone, ethylone and/or mephedrone. To confirm and quantitate these drugs, a second liquid/liquid extraction was performed using heptafluorobutyric anhydride (HFBA) as a derivatizing agent with subsequent GC/MS SIM analysis. This method was fully validated for methamphetamine, methcathionone, buphedrone, mephedrone MDA (3,4-methylenedioxyamphetamine), MDMA, methylone; and ethylone. The internal standards used were methylone-D3, MDA-D3 and mephedrone-D3.

Results: The nine urine samples that screened presumptive positive via ELISA Neogen kits for methamphetamine were confirmed positive for a synthetic cathinone. Only one sample was positive for methamphetamine, demonstrating this assay's cross reactivity to other compounds. The results of the BDS identified unique fragmentation patterns and retention times for these drugs. The study further showed that by derivatization with HFBA, a unique fragmentation and retention was produced and used to quantitative these compounds. All drugs showed a linearity of R^2>0.99 from 50-2000ng/mL with a LOQ of 50ng/mL and a LOD of 25ng/mL. The nine urine samples that tested positive for synthetic cathinones indicate that the GC/MS method can detect and quantify several bath salts. 100% of all cases tested contained methylone, 11% contained mephedrone and 22% contained ethylone. One interesting point is that in 77% of these cases were also positive for 9-carboxy-THC.

Conclusion: The Neogen ELISA kit for methamphetamine proved to be sensitive to these synthetic cathinones. The GC/MS method in both SIM and scan was developed to detect and quantify synthetic cathinones and was useful to confirm using nine urine case samples. The most prevalent synthetic cathinone seen in our population was methylone.

Keywords: Synthetic Cathinones, ELISA, Basic Drug Screen, Derivatization

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Method Development of a High Throughput Analytical Method for Cannabinoids Quantification in Blood and Urine Using LC-MS/MS

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Introduction: Quantification and detection of cannabinoids in biological matrices is extensively performed in forensic laboratories. Quantification of cannabinoids is challenging due to their high hydrophobicity and/or poor solubility in aqueous solution. Many publications report analytical methods for quantification of cannabinoids which required time consuming sample preparation and long run time chromatography for which sensitivity, recovery and matrix effect issues had been reported.

Objective: The objective of this work is to develop and validate a high throughput analytical method to quantify cannabinoids in human blood samples by protein precipitation and to qualify cannabinoids in human urine samples using dilution. Moreover, the LC-MS/MS method must be developed in order to analyse both sample types (blood and urine) using a unique LC-MS/MS chromatography.

Method: Blood protein precipitation is performed by aliquoting 100 µL of blood into a 96-well plate. An IS solution was added to each sample and plate was vortexed. Each sample was precipitated with an acetone:ACN solution, the plate was then vortexed and centrifuged. The supernatant was transferred to a second 96-well plate, diluted with acidic solution, vortexed and injected on the LC-MS/MS.

Urine dilution is performed by aliquoting 25 µL of urine into a 96-well plate. An IS solution was added to each sample and plate was vortexed. Each sample was diluted with methanol, vortexed and centrifuged. The supernatant was transferred to a second 96-well-plate, diluted with an acidic solution and injected onto LC-MS/MS.

Both extraction methods used an Agilent 1200 HPLC system using a 5.5 minutes gradient with 0.2% formic acid solution and methanol at 500 µL/min on a reversed-phase HPLC column. Detection of THC and its metabolites was performed on an AB Sciex 4000 QTrap MS using two MRM transitions per analyte. The blood calibration curve range is 0.500 to 100 ng/mL.

Results: The protein precipitation solvent and urine dilutant were selected in order to maximize cannabinoids solubility and therefore increase recoveries. Maximal solubility was obtained with 70% organic solvent. Post-column infusion was performed during chromatography optimization to minimize matrix effect at the analytes' retention time. No significant matrix effect due to ion suppression was observed for 10 ante-mortem and post-mortem matrices and recoveries of all analytes were above 70%. This method was fully validated according to ISO 17025 requirements and SWG-TOX recommendations which include selectivity, precision and accuracy, matrix effect, recovery and processed samples stability. Finally, preliminary results on adsorption of cannabinoids onto different glass and plastic surface containers observed in urine samples will be presented.

Conclusion: A high throughput LC-MS/MS method was developed and validated for cannabinoids : quantitatively in blood and qualitatively in urine samples. More investigation should be performed onto cannabinoids containers surface adsorption.

Keywords: Cannabinoids Quantification, Protein Precipitation, Urine Dilution, Adsorption

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Statistical Overview of Drug Findings in Urine Samples from the DRE Program in the Province of Quebec, Canada

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Introduction: The Drug Recognition Expert (DRE) program, although well established in the United States, is a relatively new program in the Province of Quebec. It is used since 2009 to identify subjects driving under the influence of drugs (DUID). The program consists of a 12-step evaluation to detect drug-impaired subjects who have the care and control of a motor vehicle. If drug impairment is revealed, urine samples are requisitioned and sent to the provincial forensic laboratory (LSJML) for expertise.

Objective: The main goal of this work is to present a statistical overview of the results obtained since the beginning of the DRE Program in the Province of Quebec.

Method: Over 500 urine samples from DRE cases have been sent to the laboratory since 2009. After immunological analysis, routine SPE was performed, followed by a general screening on the extract using HS-GC-FID, GC-MS/NPD and LC-MS/MS analytical methods. If necessary, confirmatory extraction and analysis were performed on a second aliquot of urine. After revision and interpretation of the case by a toxicologist, results were transferred to the LIMS and a report was sent to the police officer in charge of the case.

Results: From all cases received at the laboratory since the beginning of the program, only one was found negative (as of March 2013). Drugs in all categories covered by the program have been found, with a high frequency of cannabis, CNS depressants and CNS stimulants. Cannabis is the most commonly found drug in DRE samples. The number of methamphetamine cases has substantially increased since the beginning of the program. Methamphetamine seems to be replacing cocaine abuse which has decreased steadily over the past few years. Moreover, GHB has been found in more than 30% of DRE samples. This drug is commonly used by drivers for its recreational effects similar to alcohol without the risk of being caught by breath testing police checkpoints. Some cases of inhalants, synthetic cannabinoids, bath salts and piperazine derivatives have also been observed. Most of the cases show multi-drug abuse, involving a combination of prescription medication, over-the-counter drugs and/or illicit substances.

Conclusion: The results of our analysis identify the drugs most commonly used by DUID suspects and the high frequency of multi-drug abuse. With these statistics, it will be easier to produce a regional pattern of drug abuse and compare our results to those obtained in other Canadian provinces and in other countries.

Keywords: DRE, DUID, Statistics, Cannabis, GHB, Methamphetamine, Cocaine

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Prevalence of Synthetic Cannabinoids in Antemortem Urine Specimens Using Neogen® Corporation's Synthetic Cannabinoids Kit

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Introduction: The toxicology section of the Miami Valley Regional Crime Laboratory (MVRCL) in Dayton, Ohio is responsible for analyzing ante mortem specimens for the presence of alcohol and drugs in cases regarding human performance (e.g. driving under the influence, drug facilitated sexual assault, child endangering, etc.). In late 2010, the drug chemistry section of the MVRCL began reporting an increase in designer drugs referred to as synthetic cannabinoids, most notably JWH-018 and JWH-073. These compounds act as full agonists to the CB_1 and CB_2 cannabinoid receptors and exhibit effects similar to Δ9-tetrahydrocannabinol, the active component in marijuana.

Objective: The recent increase in synthetic cannabinoid usage has prompted the toxicology section to explore the prevalence of this designer drug class in urine cases submitted for testing.

Method: To test the population prevalence, the toxicology section employed a Synthetic Cannabinoids (SPICE) ELISA kit provided by Neogen® Corporation. In this study, 206 urine samples submitted to the MVRCL between June 2012 and March 2013 were screened for the presence of these compounds. A positive control of JWH-018 n-pentanoic acid provided with the kit was used to establish low and high positive controls at 5 and 25 ng/mL, respectively. These levels were used to determine if additional confirmation testing was warranted.

Results: The results of the ELISA screen yielded four samples (1.9%) that met criteria for supplemental testing. Three of the four samples confirmed positive for a variety of synthetic cannabinoids. While the Neogen® kit has excellent sensitivity for various JWH compounds and analogs, this study demonstrated a relatively small amount of synthetic cannabinoid presence in urine cases submitted to the MVRCL. The low rate of positive cases is most likely influenced by recent legislation implementing a national ban on many of the compounds that cross-react with this kit (e.g. JWH-018, JWH-073, JWH-200, etc.).

Conclusion: In populations where these compounds are more prevalent, this product is a powerful screening tool in the toxicology arena. Screening for the newest synthetic cannabinoids is extremely challenging due to evolving compound synthesis, changing legislation, and lack of consistent product composition. Having an open dialogue between drug chemists, toxicologists, and manufacturers of screening assays is one important step in combating this growing epidemic.

Keywords: Synthetic Cannabinoids, ELISA, Ante Mortem

P68

Effects of MAM-2201 and α-PVP on Electroencephalogram Power Spectra and Locomotor Activity in Rats

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Introduction: Synthetic cannabinoids (SCs) and cathinone derivatives (CDs) have become major classes of abused drugs worldwide. Our survey of illegal designer drugs in the Japanese market has revealed a recent trend - where mixtures of different designer drugs containing SCs and CDs are being distributed. However, pharmacological effects for SCs and CDs remain unclear.

Objective: Various kinds of psychotropic substances affect electroencephalograms (EEG) in animals and humans. We previously reported that two SCs, cannabicyclohexanol and JWH-018, changed the EEG power spectra and suppressed the locomotor activity (LOC) in rats, more significantly and for a long duration than Δ^9-tetrahydrocannabinol, a psychoactive cannabinoid of marijuana [Uchiyama, N. et al., Forensic Sci. Int., (2012) 215, 179-183]. In this study, we examined the effects of a SC MAM-2201, a CD α-PVP and a mixture of both compounds on the EEG spectra and the LOC.

Method: Sprague-Dawley male rats (8 weeks old) were implanted with EEG electrodes for polygraphic recording. After 10-days recovery, the EEG of each rat was recorded for 48 h. The first 24 h recording was used as a control with the vehicle-treatment at 10:00 a.m. The second 24 h recording was obtained after an intraperitoneal administration of MAM-2201 or α-PVP at 5 mg/kg and a mixture of MAM-2201 and α-PVP at each 2.5 mg/kg, respectively, at 10:00 a.m. The cortical EEG signal was amplified, filtered (0.5-35 Hz), and recorded by using the analysis software SLEEPSIGN. EEG spectrum was analyzed post fast Fourier transformation. LOC was measured by monitoring with an infrared device Statistical analyses were determined by Student's t-test. A P value < 0.05 was considered to be significant.

Results: The administration of α-PVP caused significantly higher LOC at each of the observed 0.5-h epochs until 6 h post administration, whereas MAM-2201 showed no statistical difference. A mixture of both compounds significantly increased the LOC at each of the observed 0.5-h epochs until 6 h, and then significantly decreased that from 10.5 h to 15 h. α-PVP and the mixture increased the total amounts of LOC to 1530% and 437%, respectively, during a 2-h period after injection. Additionally, α-PVP and MAM-2201 increased EEG power for the first 6 h in frequency ranges of 7.0-8.0 Hz and 4.0-5.5 Hz, respectively. The mixture showed EEG pattern similar to that of MAM-2201 with a slower response of up to 2 h after injection but clearly different from that of α-PVP.

Conclusion: α-PVP significantly increased the LOC and changed the EEG power spectra in rats. However, MAM-2201 did not change the LOC but significantly changed the EEG power spectra. The mixture of α-PVP and MAM-2201 significantly increased the LOC at first, and then changed the EEG power spectra with delayed response, indicating that the mixture showed the stimulant action similar to α-PVP at an early time, and subsequently changed the EEG spectra similar to MAM-2201. From the results in this study, the coadministration of α-PVP and MAM-2201 may cause more complicated serious effects than a single administration not only in animals but also humans.

Keywords: Electroencephalograms (EEG), MAM-2201

P69

Development of Synthetic Cannabinoids "SPICE" ELISA Kits for Screening of Human Urine and Blood

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Introduction: Synthetic cannabinoids are a large group of molecules that have been intentionally designed to interact with human cannabinoid receptors. The United States Drug Enforcement Agency (DEA) has designated many synthetic cannabinoids as Schedule 1 drugs under the Controlled Substance Act (21 U.S.C .812c). Specific analogs include JWH-018, JWH-073, JWH-019, JWH-200, JWH-250, JWH-081, JWH-122, JWH-398, AM2201, AM694, RCS-4, RCS-8, JWH-203, CP-47,497 and CP-47,497 C8-homolog. Europe and Australasian countries have also introduced regulations against these drugs, but kit development has been challenging because of the growing number of drugs identified in populations.

Objective: Develop ELISA kits for the detection of several select synthetic cannabinoid analogs specified in the DEA Schedule 1 list.

Method: Two haptens, JWH-018 and JWH-250, were conjugated to protein. These conjugates were immunized in rabbits and used to develop two competitive enzyme linked immunosorbent assay (ELISA) test kits.

Results: Evaluation of these polyclonal antibodies revealed the following specificity profiles: Rabbit antiserum prepared from a JWH-018-protein conjugate recognized JWH-018 as 100% cross-reactive, JWH-073 (490%), JWH-019 (94.2%), JWH-200 (612.5%), JWH-250 (0.5%), JWH-081 (6.1%), JWH-122 (51%), JWH-398 (13.2%), AM2201 (350%), AM694 (108.9%), RCS-4 (0.4%), RCS-8 (0.3%), JWH-203 (0.5%), CP-47,497 (<0.01%) and CP-47,497 C8-homolog (<0.01%). Rabbit antiserum prepared from a JWH-250-protein conjugate recognized the same list with % cross-reactivities of 32.9, 94.0, 13.6, 84.0, 100, 6.9, 9.6, 4.4, 53.4, 1.0, 0.7, 32.9, 74.5, <0.004, and <0.004, respectively. Several metabolites of these drugs were also tested. Both antisera showed cross-reactivity to select hydroxyl and carboxylated metabolites of JWH-018 and JWH-073. Only the JWH-250 antisera showed significant cross-reaction to the JWH-250 metabolites. Neither of the two antisera can detect the CP47, 497 analog series. Neither antiserum showed cross-reaction with structurally unrelated over-the-counter drugs. Dose response curves in pooled negative human urine and blood samples were spiked and compared to buffer-based control curves. The effective working range for the ELISA developed against JWH-018 is 1-40 ng/mL for JWH-018 detection in both matrices and 0.5-40 ng/mL for JWH-018 N-pentanoic acid detection in urine. The effective working range for the ELISA developed against JWH-250 for JWH-250 detection is 2-40 ng/mL and 4-40 ng/mL for blood and urine, respectively. The effective working range for JWH-018 detection is 8-40 ng/mL and 2-40 ng/mL for blood and urine, respectively. The effective working range for JWH-250 N-(5-carboxypentyl) metabolite detection in urine is 2-40 ng/mL and 5-40 ng/mL for JWH-018 N-pentanoic acid detection.

Conclusion: The data shows that these two ELISAs cross-react with the synthetic cannabinoids differently. The ELISA developed with JWH-018 antiserum has broad cross-reactivity and is particularly well suited for detection of JWH-018 and JWH-18-N-pentanoic acid metabolites in human blood and urine, respectively. The ELISA developed with JWH-250 antiserum is also broadly cross-reactive and has an improved cross-reactivity for JWH-203 and RCS-8 and is well suited for the detection of JWH-250 and JWH-250-N-pentanoic acid in human blood and urine, respectively. These two antisera will allow expanded screening of DEA Schedule 1 specified synthetic cannabinoids.

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Keywords: Synthetic Cannabinoids, Spice, ELISA, Blood, Urine

P70

Development of an ELISA for the Detection of Eszopiclone and Zopiclone in Human Urine and Blood

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Introduction: Eszopiclone and zopiclone are non-benzodiazepine hypnotic drugs. They are used in the treatment of insomnia in humans. Currently, eszopiclone is being marketed within the US as Lunesta™ and zopiclone is only available outside of the US and sold under several other brand names including Imovane™ and Zimovane™. Eszopiclone ((+)-(5S)-6-(chloropyridine-2-yl)-7-oxo-6,7-dihydro-5H-pyrrolo[3,4-b] pyrazin-5-yl 4-methyl-piperazine-1-carboxylate, CAS# 138729-47-2) is the specific (S+)-form stereoisomer found in zopiclone (CAS# 43200-80-2). Zopiclone is the racemic form of the drug containing both (S+) and (R-) form stereoisomers. Eszopiclone and zopiclone are currently classified Schedule IV under the controlled substance act in the US. Human urine and blood remain important matrix types for testing of drugs of abuse. These two drugs can be found in both matrices as parent and metabolite forms. Data from the European Medicine Agency Study 194-026 suggests concentrations of eszopiclone below 10 ng/mL in plasma have minimal effect on the EEG and cognitive impairment in humans. The point at which eszopiclone plasma concentrations fall to 10 ng/mL is approximately 8 hours following a single 3.5 mg eszopiclone dose.

Objective: Develop a highly sensitive enzyme linked immunosorbent assay (ELISA) for the detection of eszopiclone and zopiclone in human urine and blood.

Method: An antiserum was developed against an eszopiclone protein conjugate and used to develop a competitive ELISA. The assay was tested for sensitivity, cross-reactivity and matrix interference.

Results: Dose response curves in pooled negative human urine and blood samples were spiked with eszopiclone and zopiclone and compared to buffer-based control curves. The limits of detection of eszopiclone and zopiclone are 2.5-5 ng/mL for eszopiclone and 5-10 ng/mL for zopiclone in diluted human urine and blood, respectively. Human urine (n=64) and blood samples (n=51) populations containing no drugs were tested for assay interference and found to be negative at a 10 ng/mL cutoff using a 1:5 sample dilution for human urine and 1:20 for blood. Cross-reactivity was tested for zopiclone, eszopiclone, zopiclone-N-oxide, N-desmethyleszopiclone, 6-(5-chloro-2-pyridyl)-6,7-dihydro-7-hydroxy-5H-pyrrolo[3,4b]pyrazin-5-one. Cross-reactivity was found to be 100, 194.4, 100, 350.0 and 0.4% , respectively. The assay is highly specific and does not cross-react with other over-the-counter (OTC) drugs including other sleep aids such as Zaleplon and Zolpidem. The reproducibility of the dose response curves was found to be below 10%.

Conclusion: The described ELISA is robust and highly sensitive. It can simultaneously detect eszopiclone and zopiclone parent drugs and several metabolites at concentrations of 10 ng/mL in diluted human urine and blood. This assay does not cross-react with other common OTC drugs. The ELISA test kit offers a rapid and inexpensive screening option suitable for implementation in forensic testing laboratories.

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Keywords: Eszopiclone, Zopiclone, Lunesta, Zimovane, Imovane, ELISA, Blood, Urine

P71

A Six Sigma Approach to Justify Historical Calibration Use in the Postmortem Toxicology Laboratory

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Introduction: Toxicology laboratories need to operate cost effectively while producing quality results in a timely manner. This balance is complex, and the processes used must withstand scrutiny by regulatory agencies and the legal system. Six Sigma relies on DMAIC (Define, Measure, Analyze, Improve and Control); an approach used to minimize process variation so sustained defect-free performance is delivered. From an operational perspective, we examined clinical toxicology practices.

Objective: The rationale is that the clinical laboratory environment often requires quick and accurate result reporting so treatment decisions can be made. One difference between clinical and postmortem laboratories is historical calibration curve use. The forensic laboratory community has not fully embraced the use of these curves even though benefits exist including increased instrument capacity, and decreased consumable costs and labor. With this in mind, our team chose to evaluate historical curve use using DMAIC.

Method: The LC-MS/MS method for ropinirole, zolpidem and es-/citalopram was selected as a prototype, and data obtained to determine if historical calibration can be implemented without compromising result quality. Result quality for this project means that concentrations generated from real-time and historical curves consistently fall within the variability of the assay (ropinirole/zolpidem: ±20%; es-/citalopram: ±25%). To determine feasibility (the DMAIC Measure and Analyze steps) quantitative results generated for calibrators, QCs and patient samples from nine runs with n representing quantified results (ropinirole, n=113; zolpidem, n=261; es-/citalopram, n=261) were reprocessed against calibration curves that had been created and electronically stored between 1 to 12 weeks prior. Quantitative results from an additional single additional run were then reprocessed against three calibration curves created 4, 8 and 12 weeks prior, and results entered into the EP Evaluator™ (a laboratory statistics software program) using the Alternate (Quantitative) Method Comparison Module to determine if statistically identical outcomes were produced.

Results: In regard to the feasibility assessment, a comparison of the 12 week historical calibration curve against a real-time curve demonstrated good agreement (ropinirole, r^2=0.9987, y=0.9804x+1.069, n=11; zolpidem, r^2=0.9972, y=1.001x+0.946, n=23; es-/citalopram; r^2=0.9975, y=0.9997x-12.342, n=27). Statistical analysis using the EP Evaluator™ showed the following results by analyte on a run-by-run basis: ropinirole (n=10, 12, 12; r^2=0.9968, 0.9979, 0.9987, y=0.955x+1.909, y=0.977x+1.198, y=0.974x+1.196); zolpidem (n=23, 30, 22, r^2=0.9995, 0.9989, 0.9979, y=0.984x-0.7733, y=1.008x+0.6805, y=1.019x+0.9174); es-/citalopram (n=21, 37, 35, r^2=0.9946, 0.9993, 0.9998, y=0.956x-10.482, y=1.026x-7.080, y=1.013x-1.657).

Conclusion: By preparing and analyzing six less calibrators per test set-up, we project on an annual basis that instrument capacity will increase by 51 hrs, associated costs will decrease by 37% and analysts will gain 82 hrs of work time. A standard operating procedure defines and details the validation experiments and acceptability criteria that may be used at our laboratory to determine if historical calibration can be implemented for an analytical method (the DMAIC Improve step). On-going method verification is based upon the evaluation and acceptability of a calibration check sample and QC results on a run-by-run basis (the DMAIC Control step). By adhering to the Six Sigma philosophy, we put into practice a well-controlled process that can be used in the postmortem toxicology setting.

Keywords: Six Sigma, DMAIC, Historical Calibration

P72

Elimination of Drugs of Abuse in Oral Fluid from Prison Inmates Compared to Urine Samples

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Introduction: As a part of an evaluation of the possible use of oral fluid (OF) for drugs of abuse testing in Norwegian Prisons, prison inmates from 3 different prisons were enrolled in a voluntary and anonymous study. OF samples were collected on ten consecutive days after admission to the prison ward and compared to urine samples collected at the same time.

Objective: To evaluate elimination times for drugs of abuse in oral fluid, and see if OF is a viable alternative to urine for drug testing for the Norwegian correctional services (prison and probation services).

Method: 19 prison inmates participated in the study. OF and urine were sampled each morning for up to ten days and quantified by corresponding routine methods at the Norwegian Institute of Public Health using UPLC-MS/MS, LC-MS/MS or GC-MS. The samples were analyzed for amphetamines, benzodiazepines, cannabis, cocaine and opiates/opioids.

Results: 15 participants participated for 10 days, while four participants participated for 5, 7, 8 and 9 respectively. Amphetamines (11 cases), cannabis (14 cases) and different benzodiazepines (12 cases) were the most common findings. Prescribed use of methadone, buprenorphine and oxazepam is not included. The longest elimination times measured in each medium are shown in Table 1.

Table 1:

Drug	Detection time (OF)	Detection time (urine)
Amphetamine	4 days	6 days
Methamphetamine	10 days	7 days
Diazepam/Nordiazepam	10 days	10 days
Oxazepam	10 days	10 days
Nitrazepam/7-aminonitrazepam	4 days	8 days
Clonazepam/7-aminoclonazepam	10 days	9 days
Morphine	1 day	3 days
6-monoacetyl morphine	1 day	Not detected
Methadone	10 days	8 days
THC(OF)/THC-acid(urine)	2 days	10 days

Five inmates self-reported intake on admission day, 8 reported last intake 2-6 days before admission and two 7-10 days before, while two inmates reported last intake more than 10 days ago. If one takes into account the self-reported time of last intake the elimination time increases for amphetamine, methamphetamine, clonazepam, diazepam, oxazepam, morphine and 6-MAM. The value of self-reported intake can however be limited due to recollection bias.

Conclusion: OF is a good medium to uncover recent drug use; however this study shows that drug use during leave of absence for e.g. the week-end and perhaps up to a week can be covered for several substances. For cannabis and cocaine the detection times in OF are short, and a combination of OF and urine samples could therefore be the best choice. Urine will be the better choice for longer leave of absence, while OF will be suitable for follow-up after admission, random sampling and in situations in which there is a need for sampling from many inmates in a short period of time.

Keywords: Oral Fluid, Urine, Elimination Time, Drugs of Abuse, Medicinal Drugs

P73

Comparison of Liquid-Liquid Extraction and Solid Phase Extraction for the Quantitative Analysis of Opioids from Postmortem Blood

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Introduction: One liquid-liquid extraction (LLE) and two solid phase extraction (SPE) methods for the quantitative analysis of opioids (morphine, hydromorphone and oxymorphone) in postmortem blood were evaluated.

Objective: The purpose of this study was to determine if switching an existing LLE method to SPE would provide any advantages over the current procedure. The LLE procedure is a current in-house procedure. The SPE columns evaluated were Clean Screen DAU (10mL, 200mg; UCT) and Trace-B (6cc, 50mg; graciously provided by SPEWare, Inc for evaluation).

Method: LLE: Two mL of postmortem blood were spiked with 100ng of deuterated internal standards (morphine-d3, hydromorphone-d3 and oxymorphone-d3) and mixed with 2mL of acetonitrile then centrifuged. The supernatant was derivatized with 10% hydroxylamine solution for 30 minutes at 70ºC. Following a pH adjustment with 8N NaOH, analytes were extracted into 5mL of toluene:hexane:isoamyl alcohol (78:20:2 v/v/v). The extraction solvent was evaporated to dryness and derivatized with BSTFA containing 1% TMCS.

SPE-1 (CSDAU): One mL of postmortem blood were spiked with 50 ng of deuterated internal standards and mixed with 2.5mL of acetonitrile followed by centrifugation. The supernatant was evaporated to <1mL, adjusted to 3mL with 0.1M phosphate buffer (pH 6.0) and derivatized with 10% hydroxylamine solution for 30 minutes at 70ºC. SPE columns were preconditioned with methanol, water then 0.1M phosphate buffer (pH 6.0). Each sample was loaded onto an SPE column and washed with water, 0.1 M Hydrochloric acid and methanol. The columns were dried for 10 minutes. Analytes were eluted with 3mL of ethyl acetate: methanol (2:1) containing 2%: ammonium hydroxide. Extracts were evaporated to dryness and derivatized with BSTFA containing 1% TMCS.

SPE-2 (Trace-B): Two mL of postmortem blood were spiked with 100 ng of internal standards and mixed with two mL of 0.1M phosphate buffer (pH 6.0) and sonicated for 15 minutes. Samples were then derivatized with 10% hydroxylamine solution for 30 minutes at 70 ^{0}C. A pH adjustment was made with 8N NaOH followed by centrifugation. SPE columns were preconditioned with methanol then water. Each sample was loaded onto an SPE column and washed with water, 0.1 M HCl, and methanol. The columns were dried for 2 minutes at 25psi followed by drying for 1 min at 50 psi on a heated manifold. Analytes were eluted with 3mL of ethyl acetate containing 2%: ammonium hydroxide. Extracts were evaporated to dryness and derivatized with BSTFA containing 1% TMCS. GC-MS analysis was carried out using an Agilent 6890 Series GC coupled with an Agilent 5975 mass selective detector. An HP-5 GC column (25m; 0.32mm i.d; 0.17 µm film thickness). Evaluation of each procedure included five batches consisting of runs containing a calibration curve (10 to 500 ng/mL) and quality controls (75 and 300 ng/mL) for LLE and SPE-1 for SPE- 2 (30,75 and 300).A summary of the evaluations is presented in Table 1.

Results: Table 1: Method Evaluation Summary:

Extraction Method	LOQ (ng/mL)	Accuracy %	Within Run Precision %cv	Between Run Precision %cv
LLE 1	25	± 8	<8.5	<8.5
2	25	± 6	<8.0	<8.0
3	25	± 12	<10.4	<10.1
SPE-1, 1	25	± 8	<4.7	<4.4
2	25	± 8	<4.7	<4.4
3	25	± 9	<4.7	<4.1
SPE-2,1	10	± 4	<6.7	<7.4
2	10	± 3	<6.2	<6.3
3	10	± 9	<6.2	<6.0

1=Morphine, 2=Hydromorphone and 3=Oxymorphone

Conclusion: Our evaluation indicated that all procedures produced adequate sensitivity for our casework. However, the SPE-2 extraction procedure produced cleaner extracts with less interferences and was a superior method for the analysis of difficult specimens such as decomposition fluid and decomposed tissue.

Keywords: Opioids, Postmortem, Solid Phase Extraction, Liquid-Liquid Extraction

P74
Concentrations of Cardiac Drugs in Post Mortem Cases

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Introduction: The toxicology lab at the Office of the Chief Medical Examiner (OCME) of the State of Maryland routinely screens cases for the presence of five cardiac drugs: amlodipine, verapamil, diltiazem, propranolol and metoprolol. Amlodipine, verapamil and diltiazem are classified as calcium channel blockers which block L-type calcium channels. Calcium channel blockers are indicated for the treatment of hypertension, angina, arrhythmias as well as other conditions including migraines. Amlodipine is a dihydropyridine calcium channel blocker that has high vascular selectivity and is primarily used to treat hypertension. Verapamil is a non dihydropyridine (phenylalkylamine class) that is fairly selective for the myocardium and used primarily to treat angina and arrhythmia. Diltiazem is a non dihydropyridine (benzothiazepine class) that is active at both cardiac and vascular calcium channels. Metoprolol and propranolol are both beta blockers which bind to beta-adrenoceptors, blocking the binding of norepinephrine and epinephrine to these receptors. Propranolol is a non-selective beta blocker whereas metoprolol is selective for β1 receptors. Both propranolol and metoprolol are used in the treatment of arrhythmias, angina and hypertension as well as other non-cardiac related conditions.

Objective: The distribution of these drugs in heart blood, peripheral blood and liver were compiled in cases investigated by the OCME. Only one of these deaths was ruled by the medical examiner to be caused by an intoxication of the cardiac drug identified. This case was excluded from the summary table below. The cause of death for the case involved was ruled a verapamil intoxication and the verapamil concentrations were 5.7 mg/L (heart blood), 6.5 mg/L (peripheral blood) and 55 mg/kg (liver).

Method: Amlodipine, verapamil, diltiazem, propranolol and metoprolol were quantified using the alkaline drug screening procedure employed by this laboratory. Briefly, blood or tissue homogenates spiked with internal standards (mepivacaine and ethylmorphine) were extracted by liquid-liquid extraction with a back extraction. The final extract was evaporated to 200µL in isopropanol and injected into a gas chromatograph equipped with a DB-5 analytical column and a nitrogen phosphorus detector. Quantitation was performed using 2 calibrators (1.0mg/L and 4.0 mg/L for amlodipine and 0.25 mg/L and 1.0mg/L for all others). Quality controls were included with each analytical batch. All positive findings were confirmed by gas chromatography-mass spectrometry.

Results: The collected data is compiled in Table 1. There were some trends suggested by the data. Although there were some differences between heart blood and peripheral blood concentrations, these differences would not have resulted in a different interpretation of the result in most cases.

Table 1: Summary of Postmortem Cardiac Drug Concentrations (mean/median, range)

	Heart Blood (mg/L)	***Peripheral Blood (mg/L)***	***Liver (mg/kg)***	***Ht/Per Ratio***	***Liv/Ht Ratio***
amlodipine	0.12 / 0.11 0.06-0.20 n=5	0.09 / 0.09 0.07-0.10 n=2	2.4 / 1.6 0.60-4.6 n=5	2.1 / 2.1 2.0-2.1 n=2	18 / 19 6.0-31 n=5
diltiazem	1.10 / 0.40 0.02-5.30 n=8	0.41 / 0.25 0.01-1.8 n=8	5.4 / 0.90 0.05-24 n=7	1.5 / 1.3 0.50-2.9 n=7	4.5 / 3.5 2.0-10 n=6
propranolol	0.23 / 0.20 0.04-0.56 n=5	0.21 / 0.18 0.07-0.40 n=5	5.1 / 1.3 0.34-17 n=4	0.96 / 0.58 0.50-3.1 n=5	13 / 8.0 5.7-31 n=4
metoprolol	1.6 / 0.13 0.06-15 n=15	0.76 / 0.14 0.01-8.4 n=16	4.1 / 1.1 0.13-23 n=16	1.5 / 1.0 0.33-4.0 n=13	16 / 11 1.0-80 n=13
verapamil	0.47 / 0.10 0.10 – 1.2 n=3	0.70 / 0.20 0.20-1.7 n=3	4.1 / n/a 1.4-6.7 n=2	0.57 / 0.50 0.50-0.71 n=3	9.8 / n/a 5.6-14 n=2

Conclusion: There was no clear trend for heart blood or peripheral blood to have a greater concentration. It also appears that these drugs are sequestered in the liver, given the higher concentrations in the liver relative to the blood concentrations.

Keywords: Postmortem, Distribution, Cardiac Drugs

P75

Prevalence of Buprenorphine in 1300 Antemortem and Postmortem Cases in Orange County, CA Using Immunalysis® Buprenorphine Direct ELISA Kit

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Introduction: The abuse of pharmaceutical drugs has been on the rise in the USA for many years. Buprenorphine is a semi-synthetic opioid used for pain management and opioid maintenance programs. Due to its structure buprenorphine is not easily detected on any method in the Toxicology Section of the Orange County Crime Lab and is only analyzed for upon request by the submitting agency.

Objective: In order to determine if a routine screen of casework should be included for buprenorphine, a prevalence study was performed on 1300 closed cases from 2012. An Immunalysis® Buprenorphine ELISA kit was used for this study since it is able to work with the current set-up for screening in the lab for both antemortem (AM) and post-mortem (PM) casework. After screening, the samples were confirmed by LC/MS/MS analysis after a solid phase extraction.

Method: The matrix-matched standards used for this study were a 0.25 ng/mL cut-off, with a high standard of 1000 ng/mL and a blank of synthetic urine. All samples, AM and PM blood, and standards were diluted 1:10 in phosphate buffer solution (pH 7.0) prior to analysis. After mixing, the samples were analyzed on a Tecan Freedom EVO 150. For the analysis, 10 µL of each sample or standard was pipeted onto an ELISA plate. Drug conjugate, 100 µL, was added prior to 60 minute incubation at room temperature, in the dark. The wells were then washed 6 times with 350 µL of water each time and then 100 µL of 3,3',5,5' tetramethylbenzidine and urea peroxide in buffer was added to all wells. The plate was incubated at room temperature for 30 minutes before 100 µL of 1 N hydrochloric acid stop solution was added to each well. The absorbance was measured immediately at 450 nm and 650 nm and the difference between these two readings was calculated. Prior to running casework samples on the kits, standards were diluted for buprenorphine, norbuprenorphine, and the combination of the two from 0.1 – 1000 ng/mL in order to ensure that the kit worked at the cut-off concentration desired and that high dose hook effect would not occur at concentrations commonly seen in casework.

Results: Of the 1300 cases screened, 18 were deemed positive by the ELISA kit. Another 20 cases were negative, but within 30% of the cut-off, so these were also extracted for confirmation by LC/MS/MS. After LC/MS/MS analysis, 11 of the 18 cases initially found positive were confirmed and none of the initial negative cases were found to have detectable levels of buprenorphine or norbuprenorphine.

Case	Sample Type	IA Result	Buprenorphine Concentration (ng/mL)	Norbuprenorphine Concentration (ng/mL)
1	AM Blood	Pos	0.559	1.58
2	AM Blood	Pos	0.33	0.306
3	AM Blood	Pos	0.102	0.265
4	AM Blood	Pos	0.305	0.244
5	AM Blood	Pos	0.33	0.286
6	AM Blood	Pos	0.661	-
7	AM Blood	Pos	-	0.16
8	AM Blood	Pos	0.381	2.04
9	AM Blood	Pos	0.178	-
10	AM Blood	Pos	-	3.39
11	PM Blood	Pos	0.508	3.24

Conclusion: From the 1300 cases screened for buprenorphine by Immunalysis® ELISA, ~1% were positive for buprenorphine and confirmed by LC/MS/MS analysis to contain buprenorphine and/or norbuprenorphine. There were no false negatives found of the 20 samples checked. There were 7 false positives, possibly due to the confirmation method's inability to analyze for the glucuronides of both buprenorphine and norbuprenorphine.

Keywords: Buprenorphine, Prevalence Study, ELISA, LC/MS/MS

P76

Extraction Recoveries of 25 Benzodiazepines and Sedatives from Complex Matrices Using Supported Liquid Extraction with LC/MS/MS Analysis

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Introduction: The abuse of pharmaceutical drugs has been on the rise in the USA for many years. The prevalence of benzodiazepines and sedatives, poses a problem for toxicology labs analyzing driving under the influence cases as well as death investigation cases.

Objective: A fast, reliable sample extraction method that can detect many of these drugs at one time in complex matrices like urine, blood and liver, brain and stomach content homogenates is needed to facilitate laboratory workflow. The fast extraction of 23 benzodiazepines and 2 sedatives from complex matrices is successfully demonstrated using Supported Liquid Extraction (SLE). In this case, analyte recoveries were determined after extraction by LC/MS/MS.

Method: SLE in 1mL cartridge format was used to extract a 25 drug standard spiked into blank human urine, pig's blood and liver, brain, and stomach contents homogenates at concentrations ranging from 1 – 400 ng/mL. Sample pre-treatment consisted of a 1:3 dilution of spiked matrix (200µL) with 2% ammonium hydroxide (600µL). Ethyl acetate was used to elute the drugs from the columns. Extracts were evaporated to dryness, reconstituted in mobile phase and injected onto a Waters Acquity UPLC coupled to a Waters TQ-S triple quadrupole mass spectrometer.

Results: The recoveries were determined by calculating the ratio of the response of a fortified blank injected on the instrument without undergoing extraction and the response of the internal standards that were spiked into the various matrices. Ion suppression and enhancement as a function of matrix effects was determined by calculating the ratio of the response of a fortified blank from each matrix to the response of an un-extracted fortified sample in mobile phase. The matrix effects were found to be within acceptable ranges with suppression and enhancement at ± 15%.

Drug	Aqueous	Urine	Pig's Blood	Liver	Brain	Stomach Contents
7-Aminoclonazepam	90.6%	92.3%	106.1%	78.0%	81.0%	92.0%
7-Aminoflunitrazepam	92.4%	94.1%	112.1%	77.6%	79.2%	94.4%
α-Hydroxyalprazolam	93.0%	94.7%	105.9%	88.0%	79.3%	87.4%
α-Hydroxytriazolam	52.1%	47.8%	34.1%	39.2%	37.6%	48.8%
Alprazolam	76.9%	78.6%	81.2%	90.2%	81.8%	86.8%
Bromazepam	97.8%	99.5%	105.7%	83.1%	76.4%	88.6%
Chlordiazepoxide	82.6%	84.3%	74.9%	80.8%	77.6%	93.2%
Clonazepam	101.3%	103.0%	93.9%	97.5%	110.6%	101.3%
Demoxepam	79.3%	81.0%	92.7%	70.9%	66.2%	82.1%
Desalkylflurazepam	88.6%	90.3%	106.8%	75.6%	72.3%	90.9%
Diazepam	82.0%	83.7%	90.2%	98.0%	96.0%	96.7%
Estazolam	76.8%	78.5%	80.4%	85.9%	82.9%	90.3%
Flunitrazepam	41.4%	43.1%	60.4%	58.5%	53.6%	46.9%
Flurazepam	62.0%	63.7%	72.1%	78.9%	88.8%	67.5%
Hydroxyethylflurazepam	89.7%	91.4%	107.8%	73.9%	74.5%	91.6%
Lorazepam	88.0%	89.7%	80.1%	79.7%	78.6%	95.2%
Midazolam	71.5%	73.2%	80.5%	88.0%	81.8%	84.4%
Nitrazepam	83.3%	85.0%	103.1%	62.7%	62.1%	86.6%
Nordiazepam	68.9%	70.6%	71.0%	79.5%	71.5%	79.3%
Oxazepam	72.8%	74.5%	71.1%	87.8%	79.9%	83.2%
Phenazepam	94.4%	96.1%	102.6%	98.7%	101.6%	109.3%
Temazepam	72.4%	74.1%	74.7%	84.5%	78.6%	85.9%
Triazolam	98.9%	100.6%	95.4%	99.4%	94.9%	99.1%
Zaleplon	65.5%	67.2%	72.9%	74.4%	70.5%	82.7%
Zolpidem	68.6%	70.3%	77.8%	88.0%	81.6%	86.1%

Conclusion: A simple extraction method was demonstrated, which produced recoveries >65% for the majority of drugs in all six matrices at the therapeutic concentrations for all drugs.

Keywords: Sample Preparation, Benzodiazepine, LC/MS/MS

P77

Effect of Salt on Collection Time and Drug Concentration on Intercept I2 Oral Fluid Collector**

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Introduction: In recent years, interest in oral fluids as an alternate matrix for measuring drugs of abuse has increased due to the ease of collection. Sample collection is less invasive than with other bodily fluids, and adulteration is more difficult. However, individuals who are either abusing drugs or taking prescribed medications sometimes have a difficult time producing an adequate oral fluid specimen. To overcome this difficulty, stimulation of saliva flow is sometimes utilized, but this may lower the concentration of drugs with high pK values such as opiates and cocaine. Previous studies demonstrating the decreased concentration of drugs have used sour balls in combination with spitting into a tube. To our knowledge, no one has reported on the effects on drug concentrations of small amounts of salt and citric acid used in the current OraSure Intercept collector. A new collector, Intercept I2, is being developed to meet expected SAMHSA guidelines with 1mL of oral fluid collected and provide a volume indicator.

Objective: The objective of this study was to determine the amount of salt and citrate that could be applied to the pad of the Intercept I2 collection device to produce the lowest percentage of sample collection failures without impacting drug positivity rates in a high drug use population.

Method: Intercept I2 collectors were made with no treatment, medium, and high treatments of salt/citrate. 300 individual collections were performed at two high drug use population sites under IRB approval for each of these three conditions (900 total). Collection times and volumes were recorded for all sample collections. In addition 300 paired samples (600 total) were collected and were then analyzed by LC/MS/MS by previously published methods for opiates, THC, benzodiazepines and cocaine/benzoylecgonine (BE). The samples were collected no treatment followed by treated (100 pairs), treated followed by no treatment (100 pairs) and finally treated followed by treated (100 pairs). There was a ten minute wait between each of the paired collections.

Results: By increasing the salt/citrate concentration from zero to the high concentration, the percent failure of not triggering the sample adequacy indicator within 15 minutes dropped by 50% in these high drug use populations. All positive and negative predictive values were 97-100% depending on the order of collection or whether the collector pad was treated with salt/citrate or not.

Conclusion: Stimulation of saliva using salt/citrate on the collection pad of the Intercept I2 collector had no significant impact on the number of positives for Alprazolam, THC, Morphine or BE in oral fluid samples compared to an untreated Intercept I2 device using paired collections. The order of collection also had no significant impact. The use of salt/citrate on the collection pad of the Intercept I2 collector reduced the failed collection rates in high drug use populations as compared to no collector pad treatment by 50%.

Keywords: Intercept I2, Saliva Stimulation, Sequential Collections, Oral Fluid

**This collector is currently in development and has not been approved for use in the US by the FDA.
INTERCEPT is a trademark of ORASURE Technologies, Inc.

P78

Sensitive Analysis of Synthetic Cannabinoids (Spice) Compounds in Urine Using QExactive Ultra High Resolution Mass Spectrometer Collecting Full Scan and MS^2 Spectra in Data Dependent Experiment

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Introduction: The abundance of synthetic cannabinoids and the rate of new compounds appearing on the market led to specific requirements for analytical methods that provide high sensitivity, a nearly unlimited number of analytes in the method and easy addition of new analytes to the method.

Objective: Using set of spice compounds spiked into urine matrix we investigated how a new analytical workflow on QExactive™ ultra high resolution mass spectrometer collecting full scan data and MS^2 spectra in data dependent experiment will meet requirements for spice compounds analysis.

Method: Urine samples were spiked with internal standards (deuterated analogues: JWH-018 4-hydroxypentyl-D5, JWH-073 4-butanoic acid-D5), subjected to enzymatic hydrolysis and processed with SPE method. Analytes were separated in 8 min under gradient conditions using 20 mM ammonium acetate/0.1% formic acid in water as mobile phase A and 20 mM ammonium acetate/0.1% formic acid in methanol as mobile phase B. MS analysis was preformed with QExactive ultra high resolution mass spectrometer equipped with heated ESI source. Full scan data was collected with a resolution of 70K (FWHM at 200m/z) and MS^2 spectra were collected with a resolution of 17.5K. Only one MS2 scan per analyte peak was collected to allow time for MS^2 spectra collection for all coeluting analytes. Full scan data was used for quantitation and MS^2 spectra were searched against a spectral library for confirmation. Data was processed with TraceFinder™ 3.1 software.

To evaluate the analytical workflow the following spice compounds were selected: JWH-073, JWH-018, JWH-073-3-hydroxybutyl, JWH-018-4-hydroxypentyl, JWH-019-5-hydroxyhexyl, JWH-019-6-hydroxyhexyl, JWH-250-5-hydroxypentyl, JWH-018-5-pentanoic acid, JWH-073-4-butanoic acid. The calibration standards in human urine (range 0.1-20 ng/ml) and QC samples (0.2 and 1.0 ng/mL) were prepared in-house. Method precision, LOD and LOQ were obtained by processing and analyzing each calibration standard and each QC sample in 5 replicates in 3 different analytical runs. Donor samples tested positive with K-2 (Synthetic cannabinoids-1) Urine Enzyme Immunoassay specific for JWH-018, JWH-073, AM-220 and their metabolites, were confirmed using developed LC/MS method.

Results: The calibration range for all analytes was 0.1-20 ng/mL with R^2>0.99. The LODs and LOQs calculated using Linest function were between 1.5-5 ng/mL and 11-30 ng/mL respectively. Analyte peak area precision (%RSD) calculated QCs was better than 10.5%. Internal standard peak area precision was below 5%. All analytes were confirmed with MS2 spectra library search. A 100% donor samples tested positive with immunoassay were confirmed with LC/MS method for presence of JWH-18 and JWH-73 metabolites.

Conclusion: This method which collects full scan data and MS^2 spectra provided very good sensitivity and precision from the full scan data and at the same time strong confirmation with MS^2 spectra. Full scan experiment and data dependent MS^2 spectra collection allow for unlimited number of compounds in the method. Adding new compounds to the method required just m/z information, injecting the analyte on column and adding MS^2 spectra to the library.

Keywords: Spice, LC/MS, Orbitrap, Ultra High Resolution, MS^2 Spectra

P79
Withdrawn

P80
Direct Sample Analysis TOF MS of Cathinones in Bath Salts

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Introduction: Synthetic cathinones are being increasingly observed in the U.S.A as drugs of abuse, often being sold as bath salts. These synthetic stimulants are used as legal substitutes for other illicit drugs. Bath salt components are continually altered by street chemists to avoid detection. This makes law enforcement surrounding bath salts and cathinones difficult. To date, the Drug Enforcement Agency (DEA) has only successfully banned mephedrone and methylenedioxypyrovalerone (MDPV). Currently, there are no standard analytical methodologies for the detection and confirmation of cathinones in bath salts. Previously a variety of lengthy methods have been used to analyse cathinones in bath salts, including GC/MS and LC/MS/MS.

Objective: The objective of this study was to develop a rapid screening solution to rapidly detect and confirm the presence of cathinones in bath salts in seconds, without sample preparation, using Direct Sample Analysis system coupled to a Time of Flight Mass Spectrometer (DSA TOF).

Method: Cathinone standards were prepared by placing a small amount of solid sample on a mesh screen with 5 µL of methanol. Bath salt samples were directly sampled with glass tubes. The AxION DSA conditions were as follows: 5 µA corona current, 350 °C heater temperature, 80 psi auxiliary gas pressure, 4 L/min drying gas, and 25 °C drying gas temperature. The MS was run in positive ionization mode with a flight tube voltage of 8000 V. The capillary exit voltage was set to 100 and 160 V for MS and CID analyses, respectively. Mass spectra were acquired over 50–1000 m/z at 2 spectra/sec. All samples were analyzed for 15 seconds with simultaneous acquisition of lock mass.

Results: Eleven cathinone standards were analyzed by DSA/TOF and the [M+H]+ ion, as well as at least two fragment ions, were identified. The mass errors for the [M+H]+ ion in each standard were determined to be less than 5 ppm. Fragment ion identifications were made based on the losses associated with the parent ion, accurate mass, and isotopic distribution. Many cathinones shared fragment ions, such as theoretical m/z 149.0233, 126.1277, and 91.0542, corresponding to fragment ions with formulas of $C_8H_5O_3$, $C_8H_{16}N$, and C_7H_7, respectively. Isobaric cathinones such as ethcathinone and mephedrone could be separated via their fragment ion profile.

Conclusion: Five different bath salt samples (labeled numbers 3, 5, 7, 8, and 9) were analyzed and found to contain a variety of the cathinones. These were easily confirmed from previously analyzed standards. The mass spectra of samples 3 and 8 were found to contain butylone and 4-MEC, and MDPBP, butylone, α-MPPP, and α-PVP, respectively. Excellent mass accuracy was observed for real bath salt samples in only 15 sec, and no sample preparation, despite the large number of ions present. The remaining bath salt samples were found to contain: MDPV in sample 5; butylone in sample 7; and MDPBP, α-PVP, and 4-MEC in sample 9. All cathinones in the bath salt samples were confirmed by accurate mass and isotopic distribution of parent and fragment ions using AxION Solo software.

Keywords: TOF-MS, Cathinones, Bath Salts

P81

Quantitative Extraction of Commonly Prescribed Pain Medication and Drugs of Abuse in Urine Using Solid Phase Extraction (SPE) and LC/MS/MS

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Introduction: The use of pain medication for chronic pain and its gradual dependence and addiction has become a growing concern. Therefore pain medicine practitioners frequently perform urine drug testing to monitor compliance to prescribed medications and to detect substances that should not be present.

Objective: To meet the demands of the clinical market, we have developed a fast and effective analytical testing protocol for commonly prescribed pain medication and drugs of abuse. The list of drugs include amphetamines (i.e. amphetamine, methamphetamines, MDMA), benzodiazepines (i.e. alprazolam, clonazepam, diazepam), opiates (i.e. codeine, hydrocodone, hydromorphone, morphine, methadone), analgesics (i.e. tramadol, meprobamate, buprenorphine), and other drugs of abuse (i.e. phencyclidine, benzoylecgonine).

Method:

Sample Pretreatment: A 200uL urine sample was placed into a 1.5mL micro centrifuge tube and spiked with 20uL of the combined IS (Internal Standard) solution (concentrations varied from 2.5-250 ng/mL; 500-3000ng/mL for some internal standards) and vortexed for 10-15 seconds to mix. The sample was then diluted with 100uL 0.1M pH 4.0 ammonium acetate buffer and vortexed another 15 seconds. A final addition of 40uL Beta-Glucoronidase solution (100,000 units/mL) was added, followed by vortex, and then incubated for 2 hours in a shaker at 55 C to complete hydrolysis of the glucuronides. After the reaction was completed, 400ul of 0.1% formic acid was added followed by vortexing for another 60 seconds. The samples were then centrifuged at approximately 21,000 g for 10 mins (to separate any proteins).

Sample Clean Up: The supernatant from the hydrolysis procedure was loaded directly onto the Strata-X Drug B 30mg/3mL cartridges with no conditioning of the sorbent. Extracted samples were washed two times, first with 0.1% formic acid and then a 30% methanol in water. Samples were eluted using 2x500 µL of 2% ammonium hydroxide/methanol: acetonitrile (1:1). Samples were then acidified and evaporated to dryness at 40-45°C using nitrogen. Final samples were reconstituted in mobile phase and 10µL was injected onto the column.

Sample Analysis: LC-separation was achieved with a Kinetex® 2.6 µm Phenyl-Hexyl 50x4.6 or 2.1 columns using a linear gradient starting at 5% B and going to 95% B in 5min. The mobile phase consisted of 10mM ammonium formate (A) and Methanol + 0.1% Formic Acid (B). Detection was done in positive ion mode electrospray LC-MS/MS. Calibration curves were generated using a seven points from 2.5% to 1000% of the cut off ranges for the analyte, the specific range varied by analyte. Accuracy and precision were performed using QC points at 25% and 200% of the cut off value.

Results: The analytical methods provided a rapid and robust separation of all drugs of abuse analytes in a 7 minute cycle time. The Kinetex Phenyl-Hexyl also provides excellent resolution of the opiate isobaric species such as codeine and hydrocodone. The method shows excellent linearity (R^2 >0.990) and accuracy for all compounds (ranging from 99-120%). The use of SPE provides advantages over tradition “dilute-and-shoot” method for urine testing. SPE allows for sample concentration which greatly increases the sensitivity of assays and allows for older, less sensitive MS systems to be effectively used for drugs of abuse testing. The current SPE method using Strata-X Drug B shows good linearity (R^2 = 0.995) for all drugs of abuse compounds over a wide (400-fold) concentration range.

Conclusion: We have developed a simple and effective SPE clean-up method followed by LC/MS/MS analysis which can be used for the most common classes of drugs that are part of typical pain panel assays. The Strata-X-Drug B sorbent chemistry does not require conditioning and equilibration steps minimizing time and solvent consumption. The SPE method showed robustness over a dynamic range of concentrations, demonstrating high recoveries with good precision and accuracy. In addition, sample processing via SPE can greatly extend column lifetime as well as provide sample concentration to increase sensitivity. The use of the Kinetex 2.6 µm Phenyl-Hexyl provides the necessary selectivity and resolving power to analyze all target drug molecules in a total cycle time of about 7 minutes, including baseline separation of critical isobaric ions.

Reference:

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Keywords: Pain Management, Urine, Amphetamines, Benzodiazepines, Opiates, Opioids, LC/MS/MS, Solid Phase Extraction (SPE)

P82

Ultrafast Online SPE/MS/MS Screening Analysis of Bath Salts in Urine for Forensic Toxicology

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Introduction: Traditionally the screening for drugs of abuse involved analysis by immunoassays followed by a confirmatory test by GC/MS detection and more recently by LC/MS assays. The steady increase in the number of samples requiring analysis has created a bottleneck in screening for these drugs across different classes and longer turnaround times for confirmatory tests. Bath Salts – a new family of designer drugs, are synthetic cathinones with effects similar to amphetamines or cocaine. Bath salts can be modified structurally and synthesized easily, preventing them from being detected in traditional forensic drug screening. Therefore, high-throughput MS-based technologies are best suited for monitoring this class of drugs of abuse.

Objective: In the present study, we evaluated the ability of an ultrafast SPE/MS/MS system to screen for a bath salts panel in urine at low ng/ml concentrations, with sample cycle times less than 15 seconds per sample.

Method: Mass spectrometry and SPE methods were optimized on an Agilent High-throughput RapidFire Mass Spectrometry System (RapidFire 300 interfaced to an Agilent 6490 QQQ in ESI Mode). Standard calibrators were prepared by spiking drug-free urine with each drug individually and as a panel consisting of methylone (208.1→160), methcathinone (164.1→131.1), fluoromethcathinone (182.1→149.1), methoxymethcathinone (194.1→161.1), mephedrone (178.1→145.1) and methylenedioxypyrovalerone (MDPV) (276.1→205), which were then diluted, and injected for analysis. Samples were loaded onto the SPE cartridge using water with 0.09% formic acid and 0.01% trifluoroacetic acid and eluted off the cartridge using 50% methanol and 50% isopropanol with 0.09% formic acid and 0.01% trifluoroacetic acid. Sample cycle times for all compounds were under 15 seconds per sample. Data analysis was performed using MassHunter Quantitative Analysis B.05.00 software.

Results: Standard curves were prepared in a wide dynamic range by spiking a panel of six bath salts into drug-free human urine and then diluting samples 10-fold with water containing a common isotopically labeled internal standard. Each curve had excellent linearity within its measured range from 31-1000 ng/ml with R^2 values greater than 0.99. Intraday accuracies were within 15% and intraday coefficient of variation values were all less than 10% for concentrations within the measured range. Ion suppression from urine was accounted for by the use of isotopically labeled internal standards. Accurate and efficient screening of bath salts samples by SPE/MS/MS could be obtained prior to confirmation analysis of this panel by LC/MS/MS. Blinded human samples were evaluated to further verify the SPE/MS/MS methods and the results were compared to LC/MS/MS data. Strong correlations were seen between the two analytical technologies.

Conclusion: Based on these results, a panel of bath salts can be accurately, precisely and efficiently screened in urine using ultrafast SPE/MS/MS at rates of <15 seconds per sample and sensitivities at low ng/ml. This methodology is capable of throughputs of >240 samples per hour.

Keywords: Bath Salts, Urine Testing, LC/MS/MS

P83

Validation of High-Throughput Analysis of THC in Oral Fluid Using Laser Diode Thermal Desorption (LDTD) Combined with Mass Spectrometry

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Introduction: Drug testing in oral fluid is constantly evolving which benefits from increasingly sensitive methods of detection. Testing for drugs of abuse in oral fluids can strongly benefit the criminal justice field as a less invasive and cost-effective approach for drug detection when compared to blood or urine sampling.

Objective: Oral fluid analysis is an increasingly useful and non-invasive method that has facilitated laboratory analysis for many drugs of abuse. Using the Intercept device in combination with Laser Diode Thermal Desorption (LDTD) technology, we propose to validate an ultra-fast and accurate method for THC analysis in oral fluid.

Method: A calibration curve and quality control (Low, Medium and High) material are spiked into the Intercept extraction buffer (OraSure Technologies) from 1 to 1000 ng/ml. A Liquid-Liquid Extraction (LLE) procedure is performed to isolate THC before adding the extracted sample to a specially constructed 96-well plate (LazWell). The following procedure is used: 200 µL sample, 20 µL THC-d3 (1 µg/ml), 400 µL buffer and 400 µL 1-Chlorobutane. Then 4 µL of upper layer (1-Chlorobutane phase) is transferred to a 96-well plate (LazWell) and evaporated to dryness. Samples were desorbed from LazWell plate using a laser ramp of 3 seconds to a laser power of 65% and down to 0% power in 0.1 seconds. Compressed air was used as carrier gas at a flow rate of 3 L/sec. The Thermo Vantage Mass Spectrometer MS/MS transition for the THC analysis was 313->245 with collision energy of 30.

Results: The calibration curves show excellent linearity with r^2 0.9986, 0.9981 and 0.9986 for the inter-assay run. The inter-run and intra-run accuracy and precision are between 91.7 to 102.2% and 0.6 to 10.9%, respectively. Following the extraction process, all samples were stored at 4°C to evaluate the wet stability (stability of extraction mixture before LazWell plate spotting) of the drug. After 8h, all samples were re-spotted and analyzed. A wet stability of 8h is obtained with a precision and accuracy of 6.16% and 99.5% at Lower limit of Quantification (LLOQ, 1 ng/ml) standard level (n=4), respectively. The stability of dry samples in LazWell plate was also determined. All standards and QCs are spotted, dried and kept at room temperature for 48h. A dry stability of 48h is obtained with a precision and accuracy of 6.4% and 111.1% at LLOQ (1 ng/ml) standard level (n=4), respectively. Potential interfering substances (60 substances, at 10µg/ml final concentration) were added to Medium QC sample. Then the concentrations of THC were evaluated over the standard curve to evaluate the interference effect. The potential interfering substances accuracy and precision of QC are between 93.1 to 104.5% and 0.1 to 6.5%, respectively. Saliva of ten voluntaries (none THC smoker) was collected with the Intercept device for the matrix effect evaluation. Those samples were then spiked at the Medium QC level and extract to evaluate the matrix effect. The matrix effect accuracy and precision of QC are between 90.2 to 108.5% and 1.1 to 8.5%, respectively.

Conclusion: LDTD technology provides an ultra-rapid method for analysis of THC in oral fluids at 10 seconds per sample. The LDTD-MS/MS analysis is 10 times faster than a LC-MS/MS analysis having a run time of 2 minutes. The collection technique using the Intercept device coupled to LDTD-MS/MS analysis demonstrates accurate, precise and stable results for the analysis of THC in saliva.

Keywords: LDTD-MS/MS, THC, Oral Fluid

P84

High-Throughput Analysis of Cocaine in Hair Samples Using Laser Diode Thermal Desorption (LDTD) Combined with Mass Spectrometry

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Introduction: Since hair growth is fed by the bloodstream, the use of illicit drugs can be revealed by analyzing a small sample of hair. To increase the throughput analysis of hair samples, the Laser Diode Thermal Desorption (LDTD) combined with a Mass Spectrometry system were evaluated for the analysis of cocaine and benzoylecgonine (BE).

Objective: Detection and quantification of drugs in hair samples is traditionally performed by LC/MS/MS or GC/MS analysis. The Laser Diode Thermal Desorption (LDTD) system combined with Mass Spectrometry (MS/MS) allows analysis with runtimes of 10 seconds sample-to-sample. In this study, our goal was to determine the limit of detection (LOD) for cocaine and BE in hair samples using LDTD coupled with MS/MS.

Method: Negative hair matrix samples were obtained from drug-free individuals and analyzed prior to preparing matrix matched calibrators and controls. Calibrators were also spiked in blank synthetic hair matrix from UTAK® at 10 to 500 pg/mg concentration range. A liquid-liquid extraction (LLE) procedure was performed to isolate cocaine and a SPE in tip extraction procedure was used for BE before adding the extracted sample to a specially constructed 96-well plate (LazWell). The following LLE procedure was used for Cocaine: 100 µL of sample, 10 µL IS, 100 µL EDTA buffer (0.5M, pH8) and 600 µL Methyl-Tert-butyl ether (MTBE). For BE, 100 µL of sample was extracted on DPX-Polar tip and eluted with 400µL of Methanol. Then 4 µL of upper layer or elution part was transferred to a 96-well plate (LazWell) and evaporated to dryness. Samples were desorbed from LazWell plate using a laser ramp time of 3 seconds to laser power of 45%, hold for 2 seconds, and down to 0% power in 0.1 seconds. Compressed air was the carrier gas at a flow rate of 3 L/min. The MS/MS transition for the cocaine analysis was 304->182 with a collision energy of 20 and for the BE analysis the transition was 290 -> 168 with collision energy of 20, both in the positive mode.

Results: The calibration curves had excellent linearity with r^2 0.9949 and 0.9966 for the intra-assay runs, for cocaine and BE respectively. The intra-run accuracy and precision across the calibration curves were between 91.8 to 107.6% and 1.2 to 14.0% for both compounds respectively. Following the extraction process, all samples were stored at 4°C to evaluate the wet stability of the drug. After stability period, all samples were re-spotted and analyzed. A wet stability higher than 24h is obtained with a precision and accuracy between 6.0 to 12.5% and 87.7 to 99.6% at the LOD, respectively. The stability of dry samples in LazWell plate was also determined. All standards are spotted, dried and kept at room temperature for 12h. A dry stability of 12h is obtained with precision ranging from 4.3 to 10.7% and accuracy from 94.8 to 95.3% at the LOD. Six different negative hair samples were evaluated to verify the analytical method selectivity. Finally, real samples were analyzed with the LDTD-MS/MS technique and the results were compared with those obtained by the GC-MS method technique.

Conclusion: LDTD technology provides an ultra-rapid analysis method for cocaine and BE at 10 seconds per sample. The LLE or SPE in tip extraction technique combined with a LDTD-MS/MS demonstrates accurate, precise and stable results for the analysis of cocaine and BE with an LOD of 10 pg/mg.

Keywords: LDTD-MS/MS, Cocaine, Benzoylecgonine, Hair, High Throughput

P85

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

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

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

Method: All drug standards were purchased from Cerilliant Corporation (Round Rock, TX, USA). Oral fluid collection devices were purchased from Quantisal. A broad range of drugs from the pain management arena were tested including opiates, benzodiazepenes, stimulants, and hallucinogens. Over 40 drugs and their corresponding metabolites were included in this study.

DPX extractions were performed using a semi-automated extraction device to extract up to 96 samples simultaneously. The steps for sample preparation included:

1. 25ul of deuterated internal standard was added to 475 µl of stabilized oral fluid solution
2. the oral fluid solution was aspirated into a DPX-WAX tip (DPX Labs, Columbia, SC, part no. 990F) and mixed with the sorbent
3. the solution was dispensed to waste
4. the sorbent was washed by aspirating and dispensing 500 µL of water
5. analytes were eluted by aspirating and dispensing 500 µL acetonitrile

The extracts were evaporated to dryness under N_2 at ambient temperature. The extracts were then reconstituted with 175 uL 20% methanol in water. All analyses were performed using an Agilent 6410 LC/MS/MS instrument with a Poroshell EC-C18 column (3.0 x 50mm, 2.7 µm). Sample injections of 5ul were made using a 6 port (0.25mm) Cheminert C2V injection valve.

The LC mobile phase used was: A - 5mM ammonium formate with 0.05% formic acid; B – 0.05% formic acid in ACN. The gradient started at 94% A, then ramped to 100% B and held there for a total run time of 6.5 min. The flow rate was 650 µL/min.

Results: Over 40 drugs were extracted from oral fluid using the DPX-WAX tips and analyzed. These drugs included: morphine, oxymorphone, hydromorphone, codeine, amphetamine, noroxycodone, oxycodone, MDA, methamphetamine, norhydrocodone, 6 MAM, hydrocodone, O-desmethyl-cis-tramadol, MDMA, ritalinic acid, benzoylecgonine, norfentanyl, 7-amino-clonazepam, tramadol, tapentadol, cocaine, meperidine, normeperidine, meprobamate, norbuprenorphine, pentazocine, PCP, fentanyl, buprenorphine, EDDP, cyclobenzaprine, oxazepam, norpropoxyphene, lorazepam, carisoprodol, propoxyphene, 2-OH ethyl flurazepam, alprazolam, methadone, nordiazepam, and temazepam.

The DPX-WAX sorbent contains high capacity reverse phase characteristics, while maintaining the ability to form strong anion exchange properties. This permits the sorbent to bind anionic groups found in the matrix, such as peptides and proteins, while eluting the compounds of interest with acetonitrile. The unique mixing of DPX allows the extraction process to provide the highest efficiency for removing the complex sample matrices found in oral fluid.

The semi-automated DPX drug extraction and analysis method provided extraction efficiencies greater than 70% for most of the drugs screened with RSDs less than 15%. In addition, good linearity was achieved (R^2 values of 0.98 or greater) for all drugs tested. Detection limits were found to be in the range of 0.2 to 10 ng/mL for all drugs analyzed. The ranges tested, which were found to be linear, were as follows: opiates: 10 to 320 ng/ml, benzodiazepines: 4 to 128 ng/ml, amphetamines: 10 to 320 ng/ml, benzoylecogonine and cocaine: 5 to 160 ng/ml, fentanyl: 0.2 to 6.4 ng/ml, PCP: 1.0 to 32 ng/ml, buprenorphine: 2.0 to 64 ng/ml.

Conclusion: The DPX method can be used to analyze drugs in oral fluid with high recoveries. Low detection limits were achieved using a low cost LC/MS system. Most importantly, the sample preparation was non-tedious and rapid, allowing up to 96 samples to be extracted in app. 5 minutes (not including the time for solvent evaporation).

Keywords: Dispersive Pipette Extraction, Oral Fluid, LC/MS/MS

P86
Long Term Stability Study of Eight Designer Drugs in Urine

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Introduction: Designer drugs, specifically those structurally related to cathinone, have become more prevalent in case work. Many states, as well as the federal government, have passed laws making possession of these compounds illegal. Due to the increased nature of abuse and its clinical and forensic implications, a mass spectrometry method was developed to identify and quantitate these drugs of abuse.

Objective: Part of validating the method is to determine the stability of the drugs in the human biological matrix being analyzed, because the stability of the drug, in the urine, is vital to the lab's role in accurately confirming the presence and quantitation of these drugs. Quantitative data is reported in order to assist with the interpretation of toxicological results. In addition, this study provides scientific data in regards to storage of controls in urine for long term use. From this scientific data, one can predict the necessary storage conditions for specimens and calibrators of the same biological matrix.

Method: Specimen preparation is conducted using a simple dilute and shoot procedure with aqueous mobile phase. Each prepared sample is loaded onto a Waters ACQUITY® UPLC/MS/MS instrument with an HSS T3 column for the detection of eight designer drugs. The calibration range of this method is 12.5 ng/mL to 2500 ng/mL, with a cut-off concentration of 25 ng/mL. Drug free urine was spiked with all eight designer drugs at low (75 ng/mL) and high concentrations (2,000 ng/mL) and separated into two sets. One set was stored in the refrigerator, 2-8 °C, and the second set was stored in the freezer, ≤ -10 °C. An additional third set was spiked fresh each day of analysis. The refrigerator, freezer, and freshly spiked samples were analyzed on day 1, week 1 (day 8), week 2 (day 15), week 3 (day 29), month 1 (day 57), month 2 (day 85), etc., where each additional month is 28 days. To further the study of storage conditions, the refrigerator and freezer sets were stored in both amber glass bottles and clear glass test tubes.

Results: The eight drugs studied were methcathinone, mephedrone, methedrone, MDPV, pyrovalerone, methylone, ethylone, and butylone. The drug was considered stable if analysis was within 20% of its target value. The following table demonstrates the stability of each drug at 2-8 °C.

	Stability in Refrigerator (2-8 °C)					
	Day 1	**Week 1**	**Week 2**	**Week 3**	**Month 1**	**Month 2**
Methcathinone	Stable	Stable	**Not Stable**	**Not Stable**	**Not Stable**	**Not Stable**
Methylone	Stable	Stable	Stable	Stable	Stable	**Not Stable**
Ethylone	Stable	Stable	Stable	Stable	Stable	**Not Stable**
Methedrone	Stable	Stable	Stable	Stable	Stable	**Not Stable**
Butylone	Stable	Stable	Stable	Stable	Stable	Stable
Mephedrone	Stable	Stable	Stable	Stable	**Not Stable**	**Not Stable**
MDPV	Stable	Stable	Stable	Stable	Stable	Stable
Pyrovalerone	Stable	Stable	Stable	Stable	Stable	Stable
Mitragynine	Stable	Stable	Stable	Stable	Stable	Stable

All drugs were stable when stored in the freezer for up to two months. There was no difference seen for any drug when stored in amber glass bottles or clear glass test tubes.

Conclusion: In conclusion, care must be taken of how specimens, calibrators, and controls are stored in order to obtain accurate results. Monthly stability studies will continue for a total time of one year. This data will be presented at the meeting, but will only include up to eight months.

Keywords: Storage, Stability, Bath Salts, Cathinones, Designer Drugs

P87
Evaluation of the P/Tox POCT Device

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Introduction: According to national studies, illicit and prescription drug abuse remains on the rise. Point-of-care-testing (POCT) devices were introduced to clinical and forensic markets because they produce fast and accurate results with minimal training. These devices have advanced over the past five to seven years and now have the ability to test up to twelve analytes along with five specimen validity tests.

Objective: Verification of the P/Tox device involved the analysis of urine at the manufacturer's stated cutoff as well as 50% above and below the cutoff for the targeted analyte. In addition, both isomers of amphetamine and methamphetamine were analyzed at the cutoff to establish the specificity for each of the respective isomers. Common adulterants were analyzed in order to evaluate the ability of the device to identify any potential adulteration.

Method: Analytical drug standards were purchased and used to fortify drug-free urine at the three different concentrations. Additionally, the drug-free urine was utilized as the negative control. The P/Tox devices were evaluated for sensitivity, accuracy, lot-to-lot variability, and cross-reactivity. To determine the specificity of the amphetamine and methamphetamine tests, separate aliquots of drug-free urine were fortified with individual isomers and evaluated with the P/Tox device. Positive and negative results of these devices were based upon the presence of the control line along with the absence (positive result) or presence (negative result) of a test line in each drug group. Identification and quantitation of all specimens were verified using LC/MS/MS in order to ensure that the results produced via the P/Tox device were scientifically supported by a more complex analysis. Positive quality control samples at levels greater than the cutoff concentration were also evaluated in the presence of five common adulterants. The results of these tests were based upon an observed color change.

Results: The P/Tox devices produced the expected results for all of the drugs at the cutoff and 50% above the cutoff; however, some variability existed at 50% below the cutoff. No cross reactivity, which tested the target analytes against each other, was observed for any of the compounds. Furthermore, no variability between lot numbers was detected. The l-isomer of methamphetamine and amphetamine displayed negative results at the cutoff; however, the d-isomer was positive for both analytes at the same concentration. Additionally, the presence of sodium nitrite, pyridinium chlorochromate, glutaraldehyde, and bleach produced abnormal adulterant results. Only glutaraldehyde and bleach demonstrated the potential for adversely affecting the drug groups.

Conclusion: The performance of the device was evaluated by testing three different concentrations, an isomer analysis, lot-to-lot comparison, cross reactivity and an adulteration assessment. The P/Tox device demonstrated some discrepancies below the cutoff; however, all other analyses met expectations. For a rapid and user friendly presumptive test, the POCT devices are a reliable choice. Due to the potential for unexpected results, which may lead to serious medical or legal consequences, all results obtained from a POCT device should be confirmed using a more sensitive and specific methodology, such as mass spectrometry.

Keywords: Point of Care Testing (POCT), Immunoassay, Adulterations, Specimen Validity

P88

Development of a Method for the Simultaneous Analysis of Amphetamines, Cocaine and Metabolites in Urine Using DLLME and GC-MS

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Introduction: Currently it is believed that the stimulant synthetic amphetamine-type drugs (ATS) are the second class of illicit substances most used worldwide. Cocaine also assumes a prominent position and it is widely used as a drug of abuse due its stimulating effects. ATS exists in various forms, such as crystal meth (amphetamine), ecstasy (MDMA), eve (MDEA) and others. Cocaine is mainly presented in form of cocaine hydrochloride and "crack", and its use represent a serious public health problem and therefore the development of analytical methods to identify the presence of these substances in biological matrices is a topic of importance.

Objective: To develop a method for simultaneous analysis of amphetamines, cocaine and metabolites in urine samples using DLLME (Dispersive Liquid-Liquid Microextraction) and GC-MS (Gas Chromatography-Mass Spectrometry).

Method: The DLLME was performed in conical bottom tubes using 5.0 mL of urine, previously centrifuged at 3000 rpm for 10 min to remove any solid residues by precipitation. Then, the urine was fortified with a drug mix (Amphetamine, Methampethamine, Methylenedioxyamphetamine (MDA), Methylenedioxymethamphetamine (MDMA), Methylenedioxyethylamphetamine (MDEA), Cocaine, Benzoylecgonine, Cocaethylene and Anhydroecgonine methylester) at 20 ng/mL of each analyte. The sample was alkalinized using 1.0 mL of 0.1 M sodium hydroxide. 400 µL of acetone and 100 µL of chloroform were used, respectively, as dispersion and extraction solvents, homogenized in mixer for 1 min and then centrifuged. The supernatant was collected, evaporated and ressuspended in 25 µL of acetonitrile, derivatized using 25 µL of MSTFA (N-Methyl-N-(trimethylsilyl) trifluoroacetamide), incubated on a dry-block at 60°C for 30 min and then submitted to chromatographic analysis on a Varian CP-3800 gas chromatograph and a Varian Saturn 2000 GC/MS/MS mass spectrometer. Analytes were separated on a HP-5MS Capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness) Agilent Technologies®. All injections were in splitless mode with a Helium flow rate of 1.0 ml/min.

Results: The DLLME technique was able to extract all the studied analytes, resulting in clean extracts. The chromatographic analysis identified all the analytes added to the sample with good separation.

Conclusion: The developed technique was efficient to extract the analytes from samples contaminated with the drug mix at a concentration of 20 ng/mL for each analyte. We emphasize the small amount of solvent used in the extraction, about 500 microliter per sample, amount much lower when compared to traditional extraction methods.

Keywords: Simultaneous Analysis, Amphetamines, Cocaine, Metabolites in Urine

P89

Development of New Enzyme-Linked Immunosorbent Assays (ELISAs) for the Detection of the 'Z-Drugs' and Metabolites

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Introduction: The 'Z-Drugs' (zaleplon, zolpidem and zopiclone) refer to a group of medications widely prescribed as sedative hypnotics, for the short-term management of insomnia. They are known potential agents for abuse and addiction. Studies have found that users of hypnotics like the Z-Drugs had a significantly increased risk of road traffic accidents due to residual hypnotics in the bloodstream. Even more worrying is the link between zolpidem and a range of complex sleep-related behaviours, including sleep-driving. The Z-drugs have been implicated in cases of drug-facilitated sexual assault.

Objective: The aim of this study was to develop three new ELISAs for the detection of the Z-Drugs: zaleplon, zopiclone and zolpidem and metabolites in urine and blood samples. These ELISAs represent a reliable analytical tool for the screening of these drugs in test settings.

Method: Competitive colorimetric immunoassays were employed. The capture antibodies were immobilised and stabilised on the 96-well microtitre plate surface. The analyte, if present in the sample, competes with the horseradish peroxidase labelled conjugate for antibody binding sites on the microtitre plate. Absorbances were read at 450nm. The signal is inversely proportional to the concentration of drug in the sample.

Results: The zaleplon ELISA was standardised to zaleplon and the limits of detection (LODs) were 0.50 ng/ml in urine and 0.72 ng/ml in blood (calibration range: 0-220 ng/ml). The zolpidem ELISA was standardised to zolpidem and the assay also detected the major metabolite, zolpidem metabolite I (%cross-reactivity: 37%). The LODs were 0.40 ng/ml and 0.52 ng/ml in urine and blood respectively (calibration range: 0-45 ng/ml). The zoplicone ELISA was standardised to zopiclone and the assay also detected the major urinary metabolites N-desmethyl zopiclone and zopiclone-N-oxide (% cross-reactivity: 109% and 87% respectively). The LODs were 3.40 ng/ml in urine and 2.99 ng/ml in blood (calibration range: 0-500 ng/ml). The intra-assay precision (n=20), expressed as %CV, was $\leq$ 5.2% for all Z-drug ELISAs in urine and blood.

Conclusion: The Z-drug ELISAs represent the world's first immunoassays capable of the detection of the Z-Drugs: zaleplon, zopiclone and Zolpidem and metabolites in urine and blood samples. The detection of parent as well as drug metabolites in the urine increases the window of time available to corroborate the presence of these compounds. The LOD of the assays meet guidelines set out by the Society of Forensic Toxicologists (SOFT) for the detection of drugs and their metabolites in urine (recommended cut-off 10 ng/ml for zaleplon, zopiclone and zolpidem). These highly sensitive immunoassays represent a valuable tool in establishing the presence of Z-Drugs in urine and blood samples from those suspected to be involved in impaired driving and drug facilitated sexual assault offenses.

Keywords: Enzyme-Linked Immunosorbent Assays, Z-Drugs, Metabolites

P90

Development of Matrix Specific Biochip-Based Immunoassays for the Detection of Commonly Prescribed Medicinal Drugs in Whole Blood and Urine

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Introduction: Detection of commonly prescribed medicinal drugs is important in the context of workplace drugs testing, in cases of drug facilitated sexual assault (DFSA) and driving under the influence of drugs (DUID) offences. Biochip array technology increases the screening capacity, as it allows the simultaneous detection of multiple analytes from a single sample.

Objective: This study reports the analytical performance of matrix specific biochip-based immunoassays, developed for the screening of commonly prescribed medicinal drugs in both whole blood and urine. The assays detect parent compounds and metabolites. For example, immunoassays for screening of blood were developed to primarily detect methylphenidate, trazodone and fentanyl, whilst for urine the assays were developed against the metabolites ritalinic acid, trazodone metabolite (m-CPP) and nor-fentanyl.

Method: Competitive chemiluminescent biochip-based immunoassays were employed over two biochip arrays. The biochip represents a solid-state device with discrete test regions containing immobilised antibodies specific to different analytes. One biochip array enables the simultaneous screening of chloral hydrate metabolite, ethyl glucuronide, fentanyl (blood), norfentanyl (urine), ketamine metabolite, meperidine, meprobamate, flunitrazepam, zaleplon, zolpidem and zopiclone. A second biochip array enables the simultaneous screening of acetaminophen, dextromethorphan, escitalopram, ethyl glucuronide, fluoxetine, haloperidol, ibuprofen, methylphenidate (blood), ritalinic acid (urine), salicylate, sertraline, tramadol, trazodone (blood), m-CPP (urine) and tricyclic antidepressants (TCAs). The assays were applied to semi-automated Evidence Investigator analyser.

Results: The limits of detection (LOD) in whole blood and urine are shown in the table below.

Analyte	LOD (ng/ml)		Analyte	LOD (ng/ml)	
	Blood	**Urine**		**Blood**	**Urine**
Acetaminophen	4.18 ug/ml	3.98 ug/ml	Meprobamate	10.0	4.38
Chloral hydrate metabolite	0.09 ug/ml	0.18 ug/ml	Methylphenidate	8.81	N/A
Dextromethorphan	0.24	6.93	Ritalinic acid	N/A	4.02
Escitalopram	0.17	0.29	Salicylate	1.98 ug/ml	17.70 ug/ml
Ethyl glucuronide	0.09 ug/ml	0.36 ug/ml	Sertraline	1.08	0.17
Fentanyl	0.2	N/A	Tramadol	0.10	1.23
Nor-fentanyl	N/A	0.22	Trazodone	0.15	N/A
7-amino-flunitrazepam	0.4	0.68	Trazodone metabolite (m-CPP)	N/A	9.21
Fluoxetine	0.73	0.96	TCAs	2.03	4.46
Haloperidol	0.50	3.44	Zaleplon	0.4	0.6
Ibuprofen	0.91 ug/ml	12.56 ug/ml	Zolpidem	0.3	0.45
Nor-ketamine	1.9	15.33	Zopiclone	1.5	2.95
Meperidine	0.2	0.6			

The LODs for dextromethorphan, escitalopram, fentanyl/nor-fentanyl, 7-amino-flunitrazepam, fluoxetine, meperidine, meprobamate, sertraline, TCAs, zaleplon, zolpidem and zopiclone meet guidelines set out by the Society of Forensic Toxicology (SOFT), for the detection of drug-facilitated sexual assault (DFSA) drugs and their metabolites in urine. The %recovery of the analytes from spiked whole blood and urine samples at three different concentration levels, spanning the assay range, ranged from 72% to 124%. The intra-assay precision (n=20), expressed as a %CV was <18% for all assays in both matrices. The assessment of specimens showed %agreement ≥85% with other methodologies (GC/MS, ELISA).

Conclusion: The results indicate applicability of the biochip-based immunoassays to the screening of multiple commonly prescribed medicinal drugs from a single sample. The assays are sensitive and meet SOFT guidelines for DFSA drug testing where applicable. Furthermore, these immunoassays are matrix specific, which allows detection of urine or blood metabolites as well as the parent compounds. These assays have applications in workplace drugs testing where prescription medication abuse is suspected, and in the toxicology setting.

Keywords: Biochip Immunoassays, Medicinal Drugs, Multiplex Screening, Drug Testing

P91

Development of a Highly Sensitive Polyclonal Antibody for the Detection of 2,5-Dimethoxyamphetamine Designer Drugs (DOX Family)

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Introduction: The 2,5-dimethoxyamphetamines are a class of ring-substituted amphetamines which include DOB (4-bromo-2,5-dimethoxyamphetamine), DOM (2,5-dimethoxy-4-methyl-amphetamine), DON(2,5-dimethoxy-4-nitro-amphetamine) and TMA-2(2,4,5-trimethoxyamphetamine). They are psychoactive, hallucinogenic stimulants which act as serotonin 5-HT_2 receptor agonists. Most of the 2,5-dimethoxyamphetamines were developed in the 1970's and later described by the biochemist Alexander Shulgin in his compilation 'PIHKAL' in the 1990's. They became popular at that time on the illicit drug market and as a result many of these drugs became scheduled. More recently, the introduction of new substituents to the 2,5-dimethoxyamphetamine structure allows the development of novel 'legal' products which are not scheduled as controlled substances and are sold in head shops and on the internet as powders, tablets and blotters. The psychoactive properties of these designer drugs are said to range from a stimulant effect at lower doses, with hallucinogenic effects at higher doses. Reported adverse effects of these drugs include tachycardia, seizures, renal failure, self-harm and delusional psychosis. The 2,5-dimethoxyamphetamines are reputedly much more potent than traditional amphetamines so there is the risk of overdose, and as these compounds are manufactured clandestinely, they are seldom pure and are of variable strength.

Objective: The aim of this study was to develop the first highly sensitive polyclonal antibody against the 2,5-dimethoxyamphetamine (DOX family) designer drugs. This antibody could be used in the development of immunoassays for the detection of these compounds, in urine and blood samples.

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

Results: Initial evaluation of the polyclonal antibody showed sensitivity, expressed as half maximal inhibitory concentration (IC50) <4.5ng/ml (DOB as calibrator, range 0-20ng/ml; 100% cross-reactivity). The antibody showed equal cross-reactivity with DOM, >40% cross-reactivity with DON and <10% cross-reactivity with TMA-2. Amphetamine and methamphetamine were not detected.

Conclusion: The polyclonal antibody developed is highly sensitive and is suitable for the development of generic competitive immunoassays for the determination of these compounds in human biological fluids (blood, urine, oral fluid) with important forensic and toxicological applications.

Keywords: DOX Family, Polyclonal Antibody, Immunoassays, 2,5-Dimethoxyamphetamines

P92

Development of a Sensitive Polyclonal Antibody for the Detection of Adamantanylacetylindole Synthetic Cannabinoids (AB-001)

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Introduction: Since their introduction into the market place in 2004 as "legal highs", synthetic cannabinoids have rapidly increased in popularity in the United States and Europe. Originally sold under the brand name "Spice", this brand name has become a generic term to include the entire class of "legal" smoking blends sold on the internet. Dozens of cannabimimetics have been identified in herbal products and fully characterized. It is expected that many more will follow in an attempt to overcome legislation bans. One recently identified cannabimimetic is 3-[(adamantan-1-yl) carbonyl]-1-pentylindole (AB-001), which is structurally similar to AM-1248 (1-[(N-methylpiperidin-2-yl)methyl]3-adamant-1-oyl)indole and 5-fluoropentyl 5-F-AB-001. Urinary metabolites of AB-001 have been recently identified and the parent compound was reported to be absent in urine.

Objective: The aim of this study was to develop the first highly sensitive and generic polyclonal antibody against the adamantanylacetylindole family of synthetic cannabinoids, which can be used for the development of efficient immunoassays for the screening of these compounds and metabolites in human samples.

Method: The immunogen comprising AB-001 hapten conjugated to bovine thyroglobulin (BTG) as a carrier protein was administered to adult sheep on a monthly basis to provide target-specific polyclonal antiserum. IgG was extracted from the antiserum and evaluated via competitive immunoassay (ELISA).

Results: In this initial evaluation, the assay was standardised to the metabolite AB-001 N-pentanoic acid. The developed polyclonal antibody also detected the metabolite AB-001 N-pentanol (% cross-reactivity: 40%) and presented a % cross-reactivity of 7% with the parent compound. The antibody did not cross react with the first generation of cannabinoids JWH-018, JWH-073 and JWH-250 (%cross-reactivity: < 3.7%) and presented %cross-reactivity >10% with UR-144 N-pentanoic acid, UR-144 N-pentanol and XLR-11. The sensitivity, expressed as half maximal inhibitory concentration (IC_{50}) was 0.744ng/ml.

Conclusion: This initial evaluation shows the development of a highly sensitive polyclonal antibody for the detection of adamantanylacetylindole synthetic cannabinoids. The results indicate that the polyclonal antibody developed is suitable for the development of immunoassays for the generic determination of these compounds in human samples with important forensic and toxicological applications.

Keywords: Adamantanylacetylindole, Synthetic Cannabinoids, Polyclonal Antibody

P93

Development of a Highly Sensitive Polyclonal Antibody for the Detection of the Phenylacetylindole Family: RCS-8 and JWH-250

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Introduction: A number of synthetic cannabinoids, mainly belonging to the indole class, have been sold for use as recreational drugs. Such compounds, which are normally blended into a herbal smoking mixture, act as CB1 cannabinoid receptor agonists and have a psychophysiological action similar to THC. Following the identification of the first members of this drug class in 2008, a number of countries introduced legislative controls to make them illegal. More than fifty synthetic cannabinoids have been detected and identified in legal high products to date. One such compound - RCS-8 or 1-(2-cyclohexylethyl)-3-(2-methoxyphenylacetyl)indole, is a synthetic cannabinoid (also known as *SR-18* or *BTM-8)*. It can be described as an analogue of JWH-250 with the 1-pentyl group replaced by 1-(2-cyclohexylethyl), and can be expected to be less potent than JWH-250. Despite not having been reported in the scientific or patent literature as yet, reputed recreational use of RCS-8 in the United States has led to it being specifically listed in a proposed 2011 amendment to the Controlled Substances Act, which is aiming to add a number of synthetic drugs into Schedule I.

Objective: The aim of this study was to develop the first highly sensitive and generic polyclonal antibody against the phenylacetylindole family of synthetic cannabinoids – RCS-8, JWH-250 and JWH-250 metabolites. This antibody could be used for the development of a generic immunoassay for the detection of this family of synthetic cannabinoids in human urine, blood and oral fluid.

Method: The immunogen comprising RCS-8 hapten conjugated to bovine thyroglobulin (BTG) as a carrier protein was administered to adult sheep on a monthly basis to provide target-specific polyclonal antiserum. IgG was extracted from the antiserum and evaluated via competitive immunoassay (ELISA).

Results: The polyclonal antibody developed was highly sensitive for the detection of JWH-250. The sensitivity, expressed as half maximal inhibitory concentration (IC50), was <0.5ng/ml (JWH-250 as calibrator, range 0-50ng/ml; 100% cross-reactivity). The antibody also showed >25% cross-reactivity with RCS-8.

Conclusion: The polyclonal antibody developed is highly sensitive and presents cross reactivity when tested against members of the phenylacetylindole family of synthetic cannabinoids, such as RCS-8 and JWH-250. The antibody developed is suitable for the development of generic competitive immunoassays for the detection and the quantification of these compounds in human biological fluids with important forensic and toxicological applications.

Keywords: Polyclonal Antibody, Phenylacetylindole, Synthetic Cannabinoids

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First Commercially Available ELISA for the Detection of Mitragynine and its Major Metabolite in Urine and Blood Samples

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Introduction: Mitragynine is the primary indole alkaloid extracted from the leaves of the rubiaceous plant *Mitragyna speciosa*. Kratom is the name given to the leaves and tree preparations from *Mitragyna species*, which are commonly chewed, smoked or brewed as tea. Kratom has been traditionally abused in Thailand and Malaysia as a mild narcotic to increase productivity and reduce boredom. Low doses produce a cocaine-like stimulant effect whereas high doses produce an opioid-like effect. There is an emerging worldwide trend to use Kratom as a non-controlled substitute for opioid-like drugs, reflected in its demand on the internet. Krypton is the name given to a herbal preparation which contains powdered kratom leaves mixed with O-desmethyltramadol. Krypton has been associated with fatalities, illustrating that the 'herbal' blend krypton is not as harmless as it is often portrayed on internet sites.

Objective: The aim of this study was to develop the first commercially available enzyme-linked immunosorbent assay (ELISA) for the detection of mitragynine and its metabolite O-desmethylmitragynine in urine and blood samples.

Method: A competitive colorimetric immunoassay was employed. The capture antibody was immobilised and stabilised on the 96-well microtitre plate surface. The analyte, if present in the sample, competes with the horseradish peroxidase labelled conjugate for antibody binding sites on the microtitre plate. Absorbances were read at 450nm. The signal is inversely proportional to the concentration of the analyte in the sample.

Results: The assay was standardised to mitragynine, and showed 18.1% and 0.4% cross-reactivity to the metabolites O-desmethyl mitragynine and 7-hydroxy mitragynine, respectively. O-desmethyltramadol was not detected. The limit of detection (LOD) in urine was 0.71 ng/ml and in blood was 0.54 ng/ml (calibration range: 0-10 ng/ml).

Conclusion: This enzyme-linked immunosorbent assay represents the world's first immunoassay capable of the detection of mitragynine and its major urinary and blood metabolite O-desmethylmitragynine. It provides a highly sensitive and rapid analytical tool for the screening of urine and blood samples in cases of suspected mitragynine abuse. The window of detection is increased by recognition of parent as well as the metabolites. Rapid screening and subsequent confirmation of these compounds in forensic samples is critical to allow medical intervention, as fatalities have been associated with the mitragynine blend, Krypton.

Keywords: Immunosorbent Assay, Mitragynine, O-Desmethylmitragynine

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New ELISA for the Detection of New Generation Synthetic Cannabinoids: XLR-11, UR-144 and Metabolites in Urine and Whole Blood

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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. The two most common first generation synthetic cannabinoids are JWH-018 and JWH-073. UR-144 and its fluorinated version XLR-11 are the new generation of synthetic cannabinoids. UR-144 is a synthetic derivative of JWH-018 in which the naphthalene ring is substituted with a tetramethylcyclopropyl group. These new compounds are potent and addictive, and account for up to 80% of confirmed positive findings in synthetic cannabinoid-containing blood samples.

Objective: The aim of this study was to develop an ELISA for the simultaneous detection of new generation synthetic cannabinoids XLR-11, UR-144, and UR-144 metabolites in urine and blood samples.

Method: A competitive colorimetric immunoassay was employed. The capture antibodies were immobilized and stabilized on the 96-well microtitre plate surface. Synthetic cannabinoid, if present in the sample, competes with the horseradish peroxidase labelled conjugate for the antibody binding sites on the microtitre plate. Absorbances were read at 450nm. The signal is inversely proportional to the concentration of the analyte in the sample.

Results: The assay was standardized to UR-144 N pentanoic acid and showed 19% cross-reactivity to the UR-144 parent molecule. A broad range of additional UR-144 compounds were detected: UR-144-N-4-Hydroxypentyl metabolite (107%), UR-144-5-Hydroxypentyl metabolite (110%), and UR-144 desalkyl (13%). The assay also detected XLR-11 at 29% and A-834735 at 111%. The calibration range of the assay was 0-40ng/ml and the limits of detection (LOD) were 1.05 ng/ml and 0.55 ng/ml in urine and blood respectively.

Conclusion: The results obtained with this new ELISA indicate highly sensitive detection of the new generation synthetic cannabinoids XLR-11, UR-144, and UR-144 metabolites both in urine and blood. This ELISA represents a useful analytical tool for the rapid screening of urine and blood samples in cases of suspected synthetic cannabinoid abuse for these latest generation synthetic cannabinoids.

Keywords: New Generation Synthetic Cannabinoids, XLR-11, UR-144 Metabolites, ELISA

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Detection of Carboxylated Metabolites of Synthetic Marijuana in Oral Fluid

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Introduction: Synthetic cannabinoids (SC) are the most frequently found group of emerging drugs in routine testing. Like marijuana, they are ingested by smoking, produce similar subjective effects and hence are referred to as "Synthetic Marijuana". XLR-11 and UR-144 are currently the most popular but thermally unstable components in "Synthetic Marijuana" preparations. Excretion and detection of the carboxylated metabolite of marijuana in oral fluid has been reported in the past.

Objective: In this study, detection of carboxylated metabolites of both UR-144 and its pyrolytic product (UR-144 3,3,4-trimethyl pentenoyl isomer) are reported for the first time. Seventy-four oral fluid specimens previously confirmed positive (32) or negative (42) by LC/MS/MS for parent drugs XLR-11 and/or UR-144 were re-analyzed for the presence of UR-144 N-pentanoic acid, a common metabolite of XLR-11 and UR-144.

Method: Oral fluids were collected using the Quantisal device resulting in a 1:4 dilution. Standard reference materials for carboxy metabolites of UR-144 and its pyrolytic product were obtained from Cayman Chemicals. Acid metabolites for the UR-144 and its pyrolysis product were observed when human liver microsomes were incubated with commercially available XLR-11 and XLR-11 degradant respectively for a separate study. Carboxylated metabolites were extracted from 0.5 mL of acidified oral fluids (60µL phosphate buffer added, pH 1.8) by liquid-liquid extraction with hexane:ethyl acetate (7:1). The top layer was dried down and re-constituted with 50 µl methanol for a 20µL injection onto the LC/MS/MS system in negative MRM mode using ESI. Separation was performed on a 5µ biphenyl column with 0.7 mL/minute flow rate. Mobile phases were 0.1% formic acid with 2mM ammonium formate and 0.1% formic acid with 2mM ammonium formate in acetonitrile. The gradient started at 20% organic, was held for 0.5 minutes and increased to 50% , 60% and 90% after 0.6, 2.9 and 3 minutes respectively before returning to initial conditions at 4 minutes. Method was applied to 74 authentic oral fluid specimens with an administrative cut-off of 10 pg/mL.

Results: Full scan positive mode ionization MS data from microsome incubations of XLR-11 degradant showed a metabolite with the same mass and similar fragmentation to that of UR-144 N-pentanoic acid. In negative ion MRM mode, same precursor and product ions as the UR-144 N-pentanoic acid standard were present but eluted earlier than the standard. Therefore this metabolite was presumptively identified as carboxylated UR-144 degradant and later confirmed by comparison with synthesized standard. In oral fluids tested, UR-144 N-pentanoic acid metabolite was detected at or above 10 pg/mL in 15 (50%) of the 32 specimens positive for parent and 2 (5%) of the 42 negative specimens. The concentrations of the UR-144 acid metabolite ranged from 15- 500 pg/mL. The carboxylated metabolite for UR-144 degradant was also detected in all specimens positive for UR-144 N-pentanoic acid.

Conclusion: This is the first study reporting detection of carboxy metabolites of the most prevalent synthetic marijuana ingredients: XLR-11 and UR-144. Their quantitative evaluation in oral fluid opens possibilities for application as marker for SC abuse. Further research on their application and perhaps more sensitive techniques are needed to fully exploit their potential in oral fluid testing. Monitoring oral fluid for SC metabolites could prove useful in minimizing passive exposure defense in drug testing cases.

Keywords: Synthetic Cannabinoids, Oral Fluid, XLR-11, UR-144, Pyrolysis

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Stereoselective Metabolism of Venlafaxine – Fitting the Puzzle Together with Data from a Poor Metabolizer of Both CYP2C19 and CYP2D6

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Introduction: The antidepressant drug venlafaxine (VEN) is administered as the racemic mixture of the S- and R-enantiomers, which display different receptor affinities. VEN undergoes stereoselective metabolism to the major and pharmacologically active metabolite O-desmethylvenlafaxine (ODV) by cytochrome P450 (CYP) 2D6 and CYP2C19. VEN is also stereoselectively metabolized to N-desmethylvenlafaxine (NDV) by CYP2C19 and CYP3A4. It has been shown that CYP2D6 preferentially metabolizes R-VEN to R-ODV. There is no clear data in the literature on the stereoselectivity of CYP3A4 and CYP2C19 for VEN metabolism.

Objective: To gain new insight into stereoselective metabolism and elimination of VEN by comparing post mortem femoral blood S/R and metabolite/parent compound (M/P) ratios for VEN, ODV and NDV, originating from an Caucasian individual with combined poor metabolizer (PM) status of both CYP2C19 and CYP2D6, to literature values.

Method: The racemic concentrations of VEN, ODV and NDV were determined by LC-MS-MS analysis in whole blood from this single case. By an additional enantioselective LC-MS-MS analysis the S/R ratios of VEN, ODV and NDV were estimated for this case. Genotyping for CYP2C9*2,*3,*4,*5; CYP2C19*2,*3,*4,*17; CYP2D6*3,*4,*5,*6 and CYP2D6 gene copy number was performed using PCR and Pyrosequencing.

Results: The S/R ratios in post mortem femoral blood were VEN=1.1, ODV=1.8, and NDV=0.3. The concentrations of VEN, ODV and NDV were 4.5, 0.025 and 0.54 mg/kg respectively giving the M/P ratios of ODV/VEN=0.006 and NDV/VEN=0.12. Genetic analysis showed CYP2D6*4/*5, CYP2C19*2/*2, and CYP2C9*1/*1. This corresponds to PM phenotype for CYP2D6 and CYP2C19 and extensive metabolizer (EM) phenotype for CYP2C9.

Conclusion: Only one out of 700 Caucasians have combined CYP2C19 and CYP2D6 PM status therefor this case gives a rare opportunity to gain new insight. In this case, where the major metabolic pathways for venlafaxine, by CYP2C19 and CYP2D6, are non-functional other enzymes such as CYP3A4 may play a major role. The obtained M/P ratios of ODV/VEN (0.006) and NDV/VEN (0.12) were much lower than literature values and reflect the absent CYP2C19 and CYP2D6 metabolism. The found S/R ratio of ODV is considerably lower than normally found for CYP2D6 PMs, indicating that CYP2C19 preferentially O-demethylates S-VEN. The low ODV concentration reflects that only a very limited metabolism to this metabolite has taken place. Results from a previous in vitro study suggest that also CYP2C9 is involved in the formation of ODV. Given that the deceased was an EM of CYP2C9 this indicates its implication, but only of limited importance for ODV formation in vivo. The ODV S/R ratio of 1.8 indicates a stereoselective metabolism by CYP2C9. Assuming CYP3A4 is the major contributor to the formation of NDV when CYP2C19 is inactive, the low S/R NDV ratio of 0.3 indicates that R-VEN is the major substrate for CYP3A4. The abnormal S/R and M/P ratios for this individual underlines the genetic control of CYP2C19 and CYP2D6 for venlafaxine metabolism and elimination. The S/R ratios of the deceased indicate that CYP2C19 preferentially O-demethylates S-VEN, and that CYP3A4 and CYP2C9 VEN metabolism also could be stereoselective.

Keywords: Metabolism of Venlafaxine, CYP2C19 and CYP2D6

P98

Rapid Analysis of Designer Amphetamines with Fast Data Dependent Product Ion Scanning for Confirmation

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Introduction: Designer amphetamines are illicit drugs sold under various names including “bath salts”. Rapid identification and quantitation of these products is an important priority for protecting public health as well as curbing the illegal drug trade.

Objective: Liquid chromatography coupled with mass spectrometry on a triple quadrupole instrument offers sensitive and selective detection of designer amphetamines, usually using using multiple reaction monitoring (MRM) mode. We have developed an LC-MS-MS method which additionally uses data-dependent product ion scanning for on-the-fly confirmation of potential designer amphetamines.

Method: DEA exempt authentic standards for a variety of designer amphetamines were obtained from authorized sellers, and used for optimization of instrument parameters. Compounds included amphetamine, methamphetamine, MDMA, MDEA, MDAI, and many others. UHPLC was used for fast chromatographic separation with a Shimpack XR-ODS III column. A binary gradient of 0.1% formic acid and methanol was used with electrospray ionization in positive mode. The data-dependent product ion scans were triggered by detection of a peak in the MRM data channel, and were carried out at a speed of 5,000—15,000 u/sec depending on scan range.

Results: Each compound was detected using LC-MS-MS with both MRM and ultra-fast data dependent product ion scanning with spectrum matching. Calibration curves were constructed over the range of 0.25—250 ng/mL and were linear for all compounds. Limits of detection were between 0.2 and 1 ng/mL and the limits of quantitation ranged between 1 and 2 ng/mL. Three transitions were monitored for each compound to ensure transition ratios were within expected limits. This helped ensure correct identity for each peak. In addition the data dependent product ion scans, triggered on-the-fly from the MRM channels, were used for additional confirmation. The data-dependent product ion scans for each peak were averaged and searched using the software’s spectrum matching functions against a library of tandem mass spectra from authentic standards. Due to the fast scan speed of the mass spectrometer, the data-dependent product ion scans could be collected in just a few tens of milliseconds at scan speeds of 5,000—15,000 u/sec depending on scan range. No significant loss of MRM or scan sensitivity was observed when comparing fast tandem mass spectra to spectra collected at slower conventional speeds below 1,000 u/sec. The combination of MRM transition ratios as well as full product ion spectrum matching allows higher confidence to be assigned in drug screening methods.

Conclusion: A rapid and selective method for designer amphetamines incorporating ultra-fast data-dependent product ion scanning for analyte identity confirmation was developed.

Keywords: Data-Dependent MS-MS, Designer Drugs, Drug Screening, UHPLC

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Prescription Opioid Abuse. I. Profiling Oxycodone and Metabolites in Urine

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Introduction: Oxycodone (OC) is prescribed in the United States for managing moderate to severe acute or chronic pain. Although the vast majority of OC consumers are pain patients who do not misuse their medication, a large cohort of OC abusers has evolved. OC's comparable morphine-like abuse potential and widespread availability has created an epidemic of abuse, morbidity, and mortality.

Objective: Establish a database of OC and metabolite concentrations in urine following oral administration to drug-free human subjects under controlled clinical conditions.

Method: Twelve healthy, drug-free subjects (7 males/5 females) were administered a single 20 mg OxyContin® tablet (extended release) by the oral route. The study was approved by an Institutional Review Board and informed consent was obtained. Urine was collected at timed pooled intervals for 52 hrs. Specimens were frozen and shipped to the laboratory for analysis by LC-MS-MS (limit of quantitation = 50 ng/mL) for OC analytes [OC, oxymorphone (OM), noroxycodone (NOC), noroxymorphone (NOM)]. All specimens were analyzed for free drug and "total" drug following enzymatic hydrolysis.

Results: Following OC dosing, there was detectable total and free OC in 11 of the 12 subjects during the first collection period (0-2 h). The initial appearance of OC in urine was frequently accompanied by NOC. NOC was generally the most abundant metabolite and was often present in higher concentration than OC. Concentrations of total OM and NOM generally became detectable in the 2-4 h collection period. Concentrations of OC and metabolites peaked usually within 3-19 h and declined thereafter. OC was most frequently present in combination with OM, NOC, and NOM (65.4%). OC disappeared from urine before NOC and OM. Consequently, some specimens contained only metabolites late in the excretion phase (≥24 h post-dosing). As a cautionary note, in cases where OM was present (in the absence of OC), there is the potential for misinterpretation that OM was administered. Detection times (hrs, time to last positive) at a 50 ng/mL cutoff concentration in hydrolyzed urine were as follows: analyte (mean, range); OC (29.3, 24-36); OM (41.3 (24-52); NOC (39.3, 28-52); and NOM 31.8 (6-36).

Conclusion: Overall, these data suggest that drug testing requirements for OC should include tests for OC and OM in hydrolyzed urine. Use of a sensitive cutoff concentration, e.g., 50 ng/mL will enhance detection times. In addition, testing for NOC would help to distinguish OC from OM use.

Keywords: Urine, Oxycodone, Oxymorphone, Detection Times

P100

Prescription Opioid Abuse. II. Profiling Hydrocodone and Metabolites in Urine

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Introduction: Hydrocodone (HC) is a semi-synthetic opioid that is orally active as an antitussive and analgesic for the treatment of moderate to moderately severe pain. . The combination product, HC with acetaminophen, is the most frequently prescribed opioid drug with more than 136 million prescriptions dispensed in 2011 in the U.S. Because of HC's psychoactive properties, availability, and development of tampering procedures for separation from acetaminophen, HC has become one of the most commonly misused prescription drugs in the U.S.

Objective: Establish a database of HC and metabolite concentrations in urine following oral administration to drug-free human subjects under controlled clinical conditions.

Method: Twelve healthy, drug-free subjects (7 males/5 females) were administered a single 20 mg dose of HC bitartrate (two Norco® tablets) by the oral route. The study was approved by an Institutional Review Board and informed consent was obtained. Urine was collected at timed pooled intervals for 52 hrs. Specimens were frozen and shipped to the laboratory for analysis by LC-MS-MS (limit of quantitation = 50 ng/mL) for HC analytes [HC, hydromorphone (HM), norhydrocodone (NHC) and dihydrocodeine (DHC)]. All specimens were analyzed for free and "total" drug following enzymatic hydrolysis.

Results: After dosing, HC was present in 9 of 12 subject's specimens during the first collection period (0-2 h). The initial appearance of HC in urine was frequently accompanied by NHC. NHC was the most abundant metabolite and was often present in higher concentration than HC. Concentrations of total HM and DHC generally became detectable in the 2-4 h collection period. Concentrations of HC and metabolites peaked within 3-9 h and declined thereafter. HC was present in combination with HM, NHC, and DHC (34.6%). HC was not detected in some specimens. The most frequent occurrences of metabolites (in the absence of HC) were NHC alone (14.2%), HM combined with NHC (6.3%), and HM alone (0.8%). Detection times (hrs, time to last positive) at a 50 ng/mL cutoff concentration in hydrolyzed urine were as follows: analyte (mean, range); HC (27.7, 24-36); HM (25.5 (0-52); NHC (39.7, 28-52); and DHC 16.3 (0-28). Interestingly, there was unconfirmed evidence of CYP2D6 poor metabolizers in the study population. One individual produced no detectable HM and two subjects produced specimens that tested positive for HM only at very low concentrations.

Conclusion: Overall, these data suggest that drug testing requirements for HC should include tests for HC and HM in hydrolyzed urine. In addition, testing for NHC would help distinguish HC from HM use.

Keywords: Urine, Hydrocodone, Hydromorphone, Detection Times

P101
What's Up DOC?-A Case Report

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Introduction: Designer Drugs appear to be increasing in popularity due to the ease of obtaining these constituents, the lack of ability to identify the substance(s) in routine drug screening, the appeal of the drug(s) being 'safe' due to them being marketed as a 'legal high', and possibly due to stronger restrictions that are being placed on prescription drugs. As components of designer drugs are identified and regulated by the DEA, new constituents, or analogs, of these designer drugs are being manufactured to circumvent legislation. 2,5-Dimethoxy-4-Chloroamphetamine (DOC) is a substituted alpha-methylated phenethylamine, a class of compounds commonly known as amphetamines and acts as a selective serotonin receptor partial agonist. The psychedelic effects are suspected to act on the 5-HT_{2A} receptor. The strongest supposed effects include open and closed eye visuals, increased awareness of sound and movement, and euphoria. There is little literature on this particular compound and no literature that attributes death to use of this drug alone.

Objective: To present case circumstances, evidence collected at the scene and the results of the toxicology analysis so that other laboratories are aware of and can properly identify yet another compound that is being marketed as a designer drug.

Method: A blood sample collected at autopsy was sent to toxicology for analysis. Routine testing of the laboratory includes a volatile screen by gas chromatography using flame ionization detection (GC/FID), an abused drug screen by enzyme-linked immunosorbent assay (ELISA) which screens for 12 classes of drugs including amphetamines and methamphetamine and a basic/acidic/neutral drug screen by gas chromatography/mass spectrometry (GC/MS). A certified reference standard for DOC was ordered from Cayman Chemical Company in Michigan. DOC was validated for quantitation by GC/MS using MDA-d5 as the internal standard, derivatizing with heptafluorobutyric anhydride (HFBA), reconstituting with acetonitrile, and performing the analysis using a previously established GC/MS sympathomimetic amine (SMA) method and a selected ion monitoring (SIM) mode.

Results: 2,5-Dimethoxy-4-Chloroamphetamine (DOC) was identified in postmortem Iliac blood during routine basic drug analysis by GC/MS and was negative for the classes of drugs on the ELISA screen. A urine sample collected at autopsy was subjected to a routine urine spot analysis via GC/MS and the specimen was positive for DOC. Quantitation analyses showed DOC to be present at 377 ng/mL in Iliac blood; 3,193 ng/mL in Urine; 3,143 ng/g in Liver; and 683 ng/g in Brain. Gastric contents were negative for DOC.

Conclusion: This case report discusses a 37-year-old male found at home face down lying next to a psychedelic chemistry book and in the early stages of decomposition. The decedent was a known methamphetamine abuser. The evidence collected at the scene reflects that the decedent was manufacturing his version of designer drugs and then packaging them to be mailed to various places. The findings at autopsy included pulmonary edema and a subgaleal hemorrhage on the right parietal scalp. The case circumstances, evidence, and results of this case were presented so that other laboratories can be aware of another substance that is on the market. This may be the first case where death can be attributed to DOC alone.

Keywords: Designer Drug, 2,5-Dimethoxy-4-Chloroamphetamine (DOC), Sympathomimetic Amine

P102
Withdrawn

P103

Development of an Automated Homogeneous Enzyme Immunoassay for the Detection of Oxycodone in Human Oral Fluid

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Introduction: Oxycodone is a semi-synthetic opioid analgesic, which is used therapeutically in the management of moderate to severe pain. Oxycodone is metabolized to oxymorphone, an active narcotic analgesic and to noroxycodone, which is relatively inactive. Several reports reveal increased abuse of oxycodone, especially OxyContin (time-release oral oxycodone). In addition, the number of deaths linked to oxycodone consumption has increased over the past years.

Objective: Our goal was to develop a homogeneous enzyme immunoassay for the qualitative and semi-quantitative determination of oxycodone in human oral fluid. Even though immunoassays for the detection of oxycodone in urine are currently available, detection of this drug in oral fluid has a number of advantages. The collection of oral fluid is less invasive and no special facilities are required. Sample collection can be done anywhere such as at the roadside or in an office. In addition, collection can be directly monitored and there are no issues associated with the adulteration of samples. In 2004, SAMHSA proposed guidelines to use oral fluid for the detection of illicit drugs in workplace drug testing programs.

Method: Thermo Scientific CEDIA® Oxycodone Oral Fluid Assay uses two genetically engineered ß galactosidase enzyme fragments. This assay is based on the competition of the drug in the patient's sample with drug conjugated to the enzyme donor fragment (ED) of ß-galactosidase for antibody binding sites. Oxycodone present in the sample binds to the antibody, leaving the ED free to bind to the enzyme acceptor fragment (EA) and form an active enzyme. In the absence of oxycodone, the antibody binds to the oxycodone-ED conjugate, inhibiting the re-association of ED with EA. The amount of active enzyme formed and the resultant absorbance change are directly proportional to the amount of drug present in the patient's oral fluid sample.
Thermo Scientific CEDIA® Oxycodone Oral Fluid Assay qualitative mode uses a cutoff calibrator of 10 ng/mL oxycodone. The dynamic range of the assay is 0 to 50 ng/mL. The assay consists of two lyophilized reagents, two reconstitution buffers and ready-to-use calibrators and controls. The performance of the assay was evaluated on the Beckman Coulter AU680 chemistry analyzer.

Results: The within-run and total precision (CV) for the cutoff calibrator and ± 50% controls is <1.0%. The limit of detection is 1 ng/mL. No significant interference was observed from endogenous substances. A number of opiate compounds were tested to show that this test specifically detects oxycodone and oxymorphone (100% crossreactivity), but does not significantly crossreact with other structurally related molecules. Method comparison study on over 200 patient samples demonstrated good correlation between immunoassay and LC-MS/MS.

Conclusion: Thermo Scientific CEDIA® Oxycodone Oral Fluid Assay is highly specific for oxycodone and is a rapid and convenient method for the detection of oxycodone in oral fluid.

Keywords: Immunoassay, Oxycodone, Oral Fluid, CEDIA

P104
Quantitative Analysis of the NIDA 5 Panel Using Prelude SPLC System and Quantum Ultra

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Introduction: Effective October 2011, SAMHSA/NIDA guidelines allow use of LC/MS, in addition to the previously allowed GC/MS, for confirmation of samples that were initially screened positive by immunoassay. Quantitative confirmation requires six methods to correspond with the six immunoassay screening panels for analysis of THCA, opiates (morphine and codeine), amphetamines, cocaine, phencyclidine and 6-MAM (a marker for heroin). Here we developed six LC/MS/MS methods using a common sample preparation procedure, analytical column, mobile phase and instrument configuration. The methods are implemented on new Thermo Scientific™ dual channel Prelude SPLC™ liquid chromatography system which allows parallel method execution multiplexed to a single mass spectrometer. Syringe pumps used in Prelude SPLC system provide enhanced LC performance including improved peak shape and resolution, stable retention times and reduced solvent consumption.

Objective: Develop and validate simple and efficient quantitative LC/MS/MS methods for SAMHSA-compliant confirmatory analysis using novel HPLC system.

Method: A single sample preparation procedure was used for all six panels. This procedure includes enzymatic hydrolysis followed by dilution. While not all of the compounds in the panels require hydrolysis, adding this step enables all the compounds to be processed at once for subsequent separate LC/MS/MS analysis. Chromatographic separations were performed by gradient elution with a Prelude SPLC system. Separate LC/MS/MS methods were set up to analyze each of the six required panels.

The calibration standards and quality control (QC) samples were prepared by spiking compounds into blank urine. Intra- and inter- assay precision and accuracy were determined by analyzing a calibration curve with replicate QCs on three different days. Matrix effects were determined by comparing peak area of samples processed in multiple lots of urine to that of one in water. For the opiates, we were able to correlate results obtained with this method to those from a toxicology laboratory validated method. Data were processed with ion ratio confirmation.

Results: The quantitation limits for some compounds were lower than required to demonstrate method capability. The linear ranges were 2.5-2000 ng/mL for PCP and THCA; 5-2000 ng/mL for methamphetamine, BE and 6-MAM; 10-2000 ng/mL for morphine, codeine, amphetamine, MDA, and MDMA. The intra-assay precision was <13.5%, <3.5%, <14.1%, <6.9%, <9.6%, <15.9% for PCP, BE, 6-MAM, THCA, opiates and amphetamines respectively. The inter-assay precision was <8.9%, <3.6%, <10.9%, <8.8%, <7.0%, <15.3% for PCP, BE, 6-MAM, THCA, opiates and amphetamines respectively. Limited matrix effects were seen and those were largely mediated by deuterated internal standards. The percent recovery for eight spiked urine donor samples was in range of 80-120%. Data collected for opiates with developed methods correlated well with toxicology laboratory data with coefficient of correlation >0.99.

Conclusion:

- LC/MS/MS methods for 11 drugs in the NIDA-5 panel using Prelude SPLC and Quantum Ultra were developed and validated.
- Implementation of dual channel Prelude SPLC system with syringe pumps improved retention time precision, chromatographic peaks shape and resolution with reduced solvent consumption.
- Two-channel multiplexing on Prelude SPLC allows for more efficient use of the mass spectrometer which results in an average run-time of three minutes per sample.
- The methods have LOQs that satisfy the SAMSHA cutoff requirements for these 11 drugs.
- No matrix interferences were observed.

Keywords: SAMHSA, NIDA-5, LC/MS, Confirmation

P105
Quantification of 7 Designer Cathinones in Urine Using a Q Exactive Mass Spectrometer

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Introduction: Substituted cathinones, or "Bath Salts," have become the latest abused designer drugs. They are stimulants with amphetamine- and cocaine-like effects. As these drugs are not detected by current ELISA drug screening tests, new methods are needed to detect and quantitate for these compounds. While cathinones relevant concentrations are still subject of research, reported cut-off concentrations from reference laboratories are in the range of 25 ng/mL in urine.

Objective: To develop a method for MDPV, methylone, mephedrone, methedrone, ethylone, butylone and naphyrone using exact mass parent compounds for quantitation and MS2 fragments for confirmation.

Method: Samples preparation was a basified liquid-liquid extraction (LLE). During the evaporation step, DMSO was added to the test tube to prevent complete evaporation of solvent. Analytes are small and slightly volatile, and would evaporate if left too long in the evaporator. Chromatographic separation was performed using a Thermo Scientific Hypersil™ GOLD C18 column (50 x 2.1 mm, 3 µm particle size) under gradient conditions. The total run time was 5 minutes.

MS analysis was carried out on a Thermo Scientific Q Exactive™ benchtop Orbitrap mass spectrometer equipped with a heated electrospray ionization (HESI-II) probe. The Q Exactive was operated in t-MS2 mode at a resolution of 17,500 (m/z=200). Two fragments from each analyte's MS2 spectrum were selected for quantitation and confirmation. Data acquisition and processing were performed using TraceFinder software.
Validation was performed by analyzing replicate QC samples along with a calibration curve on three different days, matrix effect study and process recovery.

Results: MDPV, methylone, mephedrone, methedrone, ethylone and butylone were all linear from 0.5 to 1000 ng/mL with R2 above 0.99. Intra and Inter-assay quality control statistics demonstrate the method to be reproducible across the calibration range for the above compounds; %RSD's were better than 7.7% and 7.1% for Intra and Inter-assay respectively. Limited matrix effects were seen for the above compounds, and those were largely mediated by deuterated internal standards; recoveries of all cathinones tested in various lots of urine were in range 105-136%. Naphyrone, while it can be detected at 0.5 ng/mL, shows more variability than the other compounds (%RSD <21.4%) and a greater matrix effect from urine lot to lot (recovery >150%). A lack of a deuterated analog for naphyrone does not allow for matrix effect corrections and negatively effects method precision. Naphyrone, in this assay, should be considered qualitative.

Conclusion: We achieved our goal of a 0.5-ng/mL LOQ for the three newly-regulated cathinones, MDPV, mephedrone and methylone, as well as methylone, ethylone and butylone in urine. Naphyrone, which shows greater variability, can be detected down to 0.5 ng/mL in a qualitative manner. Deuterated internal standards are essential for rigorous quantitation of these compounds.

Keywords: Cathinones, Designer Drugs, LC/MS, HRAM, Orbitrap

P106

Direct Analysis Using Paper-Spray Mass Spectrometry: Method Development for the Rapid Screening of Drugs of Abuse for Forensic Toxicology

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Introduction: Paper spray is a direct ionization technique that simplifies the mass spectrometric analysis of dried blood spots (DBS). Paper-spray technology is therefore attractive for forensic toxicology screening for drugs of abuse. The sample collection and storage of DBS in a simple paper cassette allow shipment of samples to the forensic toxicology lab in a safe and convenient way. Both qualitative and quantitative analysis of small molecules from complex matrices such as blood or other biofluids is possible without time consuming sample preparation and chromatography.

Objective: In this work, we evaluate the analytical capability of paper spray technology coupled to a very fast and sensitive benchtop Orbitrap™ mass spectrometer for forensic toxicology screening of drugs of abuse in whole blood. Although paper spray mass spectrometry has been mostly studied for drug quantitation (1), this work hopes to determine its capability as a fast screening tool.

Method: For screening experiments (qualitative identification of drugs), mixtures of drugs were spiked in methanol/water or in blood and full MS experiments conducted for fast identification. The system (paper spray/mass spectrometry) sensitivity was assessed via a quantitative experiment, serial dilution of amitriptyline paired with its deuterated analog, as this drug has been routinely used in quantitation studies with paper spray (1). Samples were loaded onto paper cartridges and dried. Cartridges were placed in the paper spray source where a solvent is automatically introduced on to the DBS. Ions are generated directly from the paper by applying a high voltage (3-5 kV) that induces electrospray from the sharp tip of the paper. The paper-spray source was coupled to a Thermo Scientific Exactive Plus™ mass spectrometer, operated at a resolving power of 140,000 (FWHM @ m/z 200) in positive ion mode. Data acquisition used Xcalibur™ sequences with contact closure trigger from the paper spray source. TraceFinder software was used for data processing.

Results: System sensitivity was evaluated using amitriptyline/amitriptyline-d_3. Amitriptyline-spiked in solvent or in bovine blood (10–5,000 ng/mL) yielded limits of quantitation (LOQ) of 10 ng/mL for drug in solvent and LOQ of 25 ng/mL for drug in blood. In this experiment, the variability was measured in terms of %RSD (Std Dev/Mean*100) and found to be between <1 to 16% for amitriptyline detected from DBS in the concentration range of 10-5,000 ng/mL.

Drug mixtures for forensic toxicology screening were analyzed at 100, 500 and 1000 ng/mL. Amphetamine, methamphetamine, cocaine, cocaethylene, codeine and PCP were selected for the qualitative analysis. All six drugs could be identified out of blood from a full scan MS experiment, down to the 100 ng/mL level. Accurate mass m/z values were used for identification of screened drugs. Isotopic pattern matching and two fragments from an All Ion Fragmentation (AIF) experiment were used for drug confirmation.

All mass spectrometry analyses were conducted two to six hours after spotting the blood onto the paper. Inter- and intra-day variability was not rigorously studied at this time. Drug stability in DBS samples can be enhanced by storing the paper cartridges in sealed plastic bags with desiccant at room temperature and away from light.

Conclusion: These results support investigating further the coupling of paper spray to fast Orbitrap mass analyzers for use in forensic toxicology screening.

Reference:

1. Manicke, N.; Abu-Rabie, P.; Spooner, N.; Ouyang, Z.; Cooks, R.G. Quantitative Analysis of Therapeutic Drugs in Dried Blood Spot Samples by Paper Spray Mass Spectrometry: An Avenue to Therapeutic Drug Monitoring. *Journal of the American Society for Mass Spectrometry* **2011**, *22*, 1501-1507.

Keywords: Paper Spray Analysis, Forensic Toxicology Screening, Fast Screening with Orbitrap MS

P107

Validation of the Simultaneous Analysis of Heroin Addiction Treatment Compounds Using LC/MS/MS with a New Prelude SPLC System

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Introduction: There are currently several different compounds used in the treatment of heroin addiction. These compounds and their metabolites were analyzed using ThermoScientific's new SPLC system, Prelude, in combination with a TSQ Vantage mass spectrometer. The method combines a low volume SPLC system with low solvent consumption to successfully quantify methadone, 2-ethylidine-1,5 dimethyl-3,3-diphenylpyrrolidine (EDDP), buprenorphine, buprenorphine glucuronide, norbuprenorphine, norbuprenorphine glucuronide, naloxone, and naltrexone in urine.

Objective: This work verifies the validity of an SPLC-MS/MS system, as well as, a heroin treatment panel method.

Method: Chromatographic separations of all compounds were performed using ThermoScientific Prelude™ SPLC system equipped a 100x3.0, 2.6 µm particle size ThermoScientific Accucore C18 analytical column. The analytical column was kept at a constant temperature of 30°C by a ThermoScientific Multisleeve tubing heater. The system mobile phases consisted of 10mM ammonium formate, 0.05% formic acid in water and 10mM ammonium formate, 0.05% formic acid in methanol. The detector for the system was a TSQ Vantage triple quadrupole mass spectrometer with HESI-II ionization probe in positive ion mode using selected reaction monitoring (SRM). . All calibrators, quality controls, and matrix blanks were prepared in human urine at varying concentrations depending on the analyte. The samples are then diluted before injection.

Results: The system consistently performed successful chromatographic separations for the compounds of interest. Three days of accuracy and precision were performed for method verification. The interday and intraday accuracy and precision range for methadone, EDDP, naloxone, and naltrexone was evaluated from 1.0 ng/ml-1000 ng/mL. The interday and intraday accuracy and precision range for buprenorphine, norbuprenorphine, buprenorphine glucuronide, and norbuprenorphine glucuronide was evaluated from 1.00 ng/mL to 100 ng/mL. The assay precision had RSD values that were less than 15.0% for all compounds tested. Additionally, accuracy was ±15% of the theoretical value for all the analytes. The correlation coefficient values for all the calibration curves ranged from 0.9945 to 0.9994. Every compound passed criterion for recovery (>90%), carryover, specificity, benchtop stability and autosampler stability. Isobaric and other possible interferences were separated chromatographically.

Conclusion: A method for heroin addiction treatment was validated. The method is five minutes from injection to injection. However, the Prelude SPLC is capable of multiplexing two HPLC channels to a single mass spectrometer reducing run time by diverting the flow from one HPLC stream (when no compounds are eluting), while the second HPLC stream elutes into the mass spectrometer. The injections are off set in time such that only one HPLC channel is eluting compounds of interest at any given time. Therefore, the total sample run times are 2.5 minutes per sample when multiplexed. The Prelude SPLC uses a single syringe fill per sample, which provides several improvements over traditional HPLC systems. These enhancements reduce the mechanical wear and tear on the pumps, and remove the need for pulse dampeners, proportioning valves, and active check valves. The result is a more robust system that requires less maintenance; reducing cost and down time compared to conventional HPLC systems. Validation of a method for heroin addiction treatment has been developed on a new online cleanup, Prelude™ sample preparation liquid chromatography mass spectrometer (SPLC-MS). Prelude's low volume, low solvent consumption saves time and resources compared to traditional HPLC systems and are designed to be more robust and require less maintenance.

Keywords: Chromatographic Separations, Heroin Treatment Panel, HPLC

P108

Identification and Quantitation of Muscimol and Ibotenic Acid in *Amanita Gemmata* Mushrooms

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Introduction: Mushrooms of the *Amanita* genus produce several toxic alkaloids, chief among them are muscimol and ibotenic acid. Muscimol, a potent $GABA_A$ receptor agonist, has psychoactive and dissociative properties. In addition to being a prodrug for muscimol, ibotenic acid can cause excitotoxic lesions in the brain. Both compounds are readily absorbed after oral ingestion of *Amanita* mushrooms, which can occur accidentally or recreationally. Levels of muscimol and ibotenic acid in *Amanita muscaria* have been reported in the literature and can vary widely.

Objective: A lesser-known species, *Amanita gemmata*, grows in the Pacific Northwest and is thought to contain muscimol and ibotenic acid. A GC/MS/MS method was developed to quantitate the muscimol and ibotenic acid in extracts of locally harvested *A. gemmata*. The method must be compatible with a larger drug screen that is routinely used in the laboratory.

Method: Sections of dried mushroom cap were ground to a fine powder with a mortar and pestle. 5 milligrams of mushroom powder was weighed out and dissolved in 2 mL of pure methanol. Phenacetin, the internal standard, was added. The mixture was shaken at room temperature for 10 minutes and centrifuged at 3000 rpm for 5 minutes. The supernatant contained the compounds of interest. 150 microliters of extract was dried down under nitrogen, at room temperature, and then reconstituted in 50 microliters of ethyl acetate. BSTFA containing 1% TMCS was used as a derivitizing reagent. The reaction proceeded at 37 degrees C for 20 minutes. The extracts were transferred to autosampler vials. A robust GC/MS/MS MRM method was developed on a Bruker Scion TQ instrument. Two transitions were monitored for each compound. The method was calibrated in the range of 100 nanograms/milliliter to 5 micrograms/milliliter.

Results: Two samples of dried *Amanita gemmata* were received as evidence from a possible poisoning case in the Sacramento area and were tested for ibotenic acid and muscimol. Levels are reported as the average of three independent extractions. Both mushrooms came from the same location but were harvested at different times.

Sample	Muscimol (ng/mg tissue)	Ibotenic Acid (ng/mg tissue)
A. gemmata 1	1194 ± 137	492 ± 96
A. gemmata 2	1628 ± 131	1548 ± 198

Conclusion: The method presented here allows extraction and quantitation of muscimol and ibotenic acid from dried mushroom tissue, including that of *Amanita gemmata*. The levels seen are likely high enough to result in intoxication after consuming *A. gemmata*, though the ratio of ibotenic acid and muscimol may vary with location and maturity of the mushroom. In the future we plan to expand our method to include other mushroom toxicants and develop extraction methods for biological samples in cases of poisoning or accidental ingestion.

Keywords: GC/MS/MS, Amanita, Hallucinogenic Mushroom

P109

Development and Validation of Methods for Compounds of Interest in Therapeutic Drug Monitoring on the New Prelude SPLC LC-MS/MS System

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Introduction: Bioanalysis using LC-MS/MS can be difficult due to complex sample preparation and variability from sample handing. In addition, both immunosuppressant and chemotherapeutic drugs often have a narrow therapeutic range and require accurate monitoring to avoid toxic events from over dosing or lack of efficacy from under dosing. The use of liquid chromatography-tandem mass spectrometry (LC-MS/ MS) to quantify the immunosuppressant drugs cyclosporine A, serolimus, tacrolimus, and everolimus, and the chemotherapeutic drugs busulfan, methotrexate, imatinib, and docetaxel is common practice.

Objective: We demonstrate the application of Prelude SPLC™ system in developing a faster, more reproducible and lower solvent consuming methods for measuring immunosuppressant and chemotherapeutic drugs.

Method: The immunosuppressants were prepared in human whole blood while the chemotherapeutics were prepared in human plasma. Online sample cleanup by turbo flow technology and analytical separation was performed on a new Prelude™ sample preparation liquid chromatography (SPLC) system. Detection of eluting analytes was performed with a TSQ Vantage™ triple stage quadrupole mass spectrometer, equipped with a heated electrospray ionization (HESI II) probe in positive ion mode using selected reaction monitoring (SRM). All methods were dual column methods using TurboFlow columns for online sample clean-up. The immunosuppressant used a Cyclone P 0.5 x 50 mm TurboFlow column and the chemotherapeutics used a C18 XL column. An Accucore C8 2.1 x 50 mm, 2.6µ column was the analytical column used for immunosuppressants while an Accucore C18 2.1 x 50 mm, 2.6 µ column was used for busulfan, methotrexate, imatinib, and docetaxel. The mobile used in all experiments excluding docetaxol were 10mM ammonium formate, 0.05% formic acid in methanol and in water. Docetaxel used 0.1% formic acid in water and acetonitrile. Quantitation was calculated with Thermo Scientific LCquan™ software. The total run times were less than four minutes per sample. However, the Prelude SPLC is capable of multiplexing two HPLC channels to a single mass spectrometer reducing runs time by diverting the flow from one HPLC stream (when no compounds are eluting), while the second HPLC stream elutes into the mass spectrometer. The injections are off set in time such that only one HPLC channel is eluting compounds of interest at any given time. Therefore, the total sample run times are <2 minutes per sample when multiplexed. The methods consumed less than 3 mL of mobile phase per injection.

Results: The validated method ranges for this study were 1-50 ng/mL for Serolimus, Tacrolimus, and Everolimus, 10-2000 ng/mL for Cyclosporine A, 1-2000 ng/mL for busulfan, 10-2000 ng/mL for imatinib, 3-1000 ng/mL for docetaxel, and 10-750 ng/mL for methotrexate. Individual compounds were evaluated for both inter and intra-day accuracy and precision, recovery, carryover, specificity, bench top and auto sampler stability, and matrix effects. All the calibrators and controls were within ±15% of the expected concentration. The standard curves had correlation coefficients between 0.991 and 0.999. No stability issues were observed. Recoveries including matrix effects ranged from 90-110%. All compounds passed specificity (no interferences from blank matrix) and carryover criterion (<10% of LLOQ from blank following ULOQ).

Conclusion: Methods for the chemotherapeutics have been reported previously using liquid-liquid extraction and longer HPLC methods (7-15 minutes); however, utilizing the new SPLC system reduced the method run to under 4 minutes. The shorter method time allows for higher throughput sample analysis, which is increased further by the Prelude's multiplexing capabilities. The ability of using less solvents leads to reduction in cost. On-line sample clean-up, also lowers the sample preparation duration and variability of the methods by minimizing sample handling. The Prelude SPLC uses a single syringe fill per sample that provides several improvements over traditional HPLC systems. These enhancements reduce the mechanical wear and tear on the pumps, and remove the need for pulse dampeners, proportioning valves, and active check valves. The result is a more robust system that requires less maintenance; reducing cost and down time compared to conventional HPLC systems.

Keywords: Chemotherapeutics, Immunosuppressants, LC/MS, Quantitation, SPLC, On-Line Clean-Up, TurboFlow

P110

Development and Verification of a Drug Panel for Simultaneous Screening and Conformation of Drugs of Abuse and Pain Management on the New Prelude SPLC LC-MS/MS System

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Introduction: Advances in both mass spectrometry (MS) and high pressure liquid chromatography (HPLC) have improved the speed, sensitivity, and accuracy of LC/MS methodologies. Large panels of compounds can be simultaneously quantified with a single injection in a few minutes.

Objective: In this study we incorporated the new TSQ Vantage™ triple stage quadrupole mass spectrometer, with a Prelude SPLC™ that is more robust and reproducible than traditional HPLC systems. A panel of over 40 drugs including, but not limited to, benzodiazapenes, opiates, drugs of abuse, and pain management was verified for screening and quantification from a single injection. All the compounds met acceptance criterion for verification.

Method: Human urine samples were spiked with each compound and select compound metabolites. Isotopically labeled internal standards were then added to each aliquot. Enzymatic cleavage of the glucuronides was performed by incubation with glucuronidase at 60°C for 2 hours. After cleavage, the incubations were quenched with methanol. Samples were then diluted further with water to get into the operating range of the mass spectrometer. Two SRM transitions were monitored for all compounds, one for quantitation and one for conformation. An Accucore aQ 2.1 x 100 mm, 2.6 column was used for separation of isobaric interferences. The mobile phases were 10mM ammonium formate, 0.05% formic acid in methanol and in water. Gradient elution over 8 minutes was used with a total run time injection to injection of 10 minutes.

Results: Individual compounds were evaluated for accuracy, precision, recovery, carryover, specificity, possible exogenous interferences, room temperature and autosampler stability, and matrix effects. All the calibrators and controls were within ±15% of the expected concentration. The standard curves had correlation coefficients between 0.990 and 0.999. No stability issues were observed. Recoveries, including matrix effects, ranged from 90-110%. All compounds passed specificity (no interferences from blank matrix) and carryover criterion (<20% of LLOQ from blank following ULOQ).

Conclusion: The verification of a method to both screen and quantify a large panel of compounds for Drugs of Abuse (DOA) and Pain Management was achieved with a Prelude SPLC system attached to a TSQ Vantage triple quadrupole mass spectrometer. The method is 10 minutes long and separates all the isobaric interferences chromatographically. All the compounds tested pass acceptance criterion for accuracy, precision, recovery, carryover, matrix effects, specificity and short term stability of samples in matrix and after sample preparation. Long term stability of stored samples was not tested. The method can be broken down by class if shorter method run times (>5 minutes) are necessary. For example, if only the opiates are screened the method can be reduced to 4 minutes (only the part of the gradient where the opiates elute is necessary). The Prelude SPLC is capable of multiplexing two HPLC channels to a single mass spectrometer reducing runs time by diverting the flow from one HPLC stream (when no compounds are eluting), while the second HPLC stream elutes into the mass spectrometer. The injections are off set in time such that only one HPLC channel is eluting compounds of interest at any given time.

Keywords: LC/MS, Drugs of Abuse, Pain Management, SPLC, Prelude, Screening, Quantification

P111

Detection of Ethylglucuronide/Ethylsulfate in Urine Samples Collected in Sexual Assault Cases

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Introduction: Of all the analyses carried out by forensic toxicology laboratories, the determination of ethanol is probably the most common test. As most forensic toxicology laboratories measure parent and metabolite concentrations in biological samples, the measurement of the metabolites of ethanol is rarely performed. In this study, samples of urine taken from closed out cases (ethanol related sexual assault) were analyzed for the concentrations of ethylglucuronide (EtG) and ethylsulfate (EtS) using solid phase extraction and LC-MS/MS.

Objective: This study was initiated to develop a simple, robust method that would assist forensic analysts in the analysis of EtG/EtS in urine samples. The method is based on the employment of anion solid phase extraction (SPE) to produce clean samples for analysis by liquid chromatography-tandem mass spectrometry. The developed method was then applied to genuine (DFSA) cases to determine both EtG/EtS concentrations where the ethanol concentration was negative.

Method: SPE of urine samples (Calibrators, controls, and test) was performed on anion exchange columns pre-conditioned with methanol, deionized water (3mL, 3mL, respectively) prior to sample loading. 1.0 mL urine samples (containing deuterated analogues of EtG/EtS) were diluted with 4mL of de-ionized (DI) water and mixed. After loading the samples onto the SPE columns, the cartridges were washed with DI water, and methanol (3 mL of each, respectively). Each SPE column was dried and eluted with 2 x 3 mL of methanol containing 6% acetic acid. The samples were then evaporated to dryness and the residues dissolved in 100 µL of mobile phase for analysis by LC-MS/MS in negative multiple reaction monitoring (MRM) mode. Data is presented for MRM's employed (3 each for: EtG/EtG-D5, EtS/EtS-D5). Liquid chromatography was performed in isocratic mode employing a 100 mm x 2.1 mm (4µm) silica hydride analytical column and a mobile phase consisting of acetonitrile and 0.1% aqueous formic acid (50% acetonitrile) at a flowrate of 0.35 mL/ minute.

Results: The limits of detection for this method were determined to be 10ng/ mL for EtG/EtS. The method was found to be quantitatively linear from 25 ng/ mL to 500 ng/ mL (r^2>0.999). Recoveries were determined to be >95% for all of the compounds. Interday and Intraday analysis of the EtG/EtS were found to < 8% and < 10 %, respectively. Matrix effects were determined to be < 6%. Concentrations of EtG and EtS are presented in 15 genuine (completed) cases (ranges: 100 to >500 ng/ mL).

Conclusion: As the forensic community becomes more aware of EtG/EtS as measureable metabolites, the use of this procedure for the analysis of EtG/EtS will be of great use to analysts in the field of forensic urine analysis as it demonstrates the use of SPE/LC-MS/MS to provide data on EtG/EtS in sexual assault cases where the ethanol concentrations in this medium are low (i.e.below the laboratory cut off threshold) or negative. EtG/EtS detection above a certain concentration is confirmation that ethanol was consumed however; correlation to an ethanol concentration is not possible.

Keywords: ETG, SPE, DFSA

P112
Determination of Pesticide Residues in Marijuana by QuEChERS and LC-MS/MS

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Introduction: Marijuana is one of the most highly abused drugs in the world. Although the concentrations of active ingredient (THC) may be determined by laboratories, few studies have looked at possible organic contaminants such as pesticides in seized marijuana samples. By developing a robust and efficient method using QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) and LC-MS/MS techniques, the identities and concentrations of 23 pesticides, including methamidophos, carbendazim, dicrotophos, acetachlor, thiabendazole, DIMP, tebuthiuron, simazine, carbaryl, atrazine, DEET, pyrimethanil, malathion, bifenazate, tebuconazole, cyprodinil, diazinone, zoxamide, pyrazophos, profenofos, chlorpyrifos, abamectin, and bifenthrin were determined.

Objective: This project was developed to determine the identities and concentrations of pesticides in street level marijuana using a robust and efficient methodology. In this study QuEChERS method was employed in conjunction with LC-MS/MS for pesticide analysis.

Method: To each calibrator and test sample (2g) was added 10 mL of deionized (DI) water. Samples were mechanically shaken for 60 minutes, after which the internal standard (triphenyl phosphate (1 µg)) was added. To the samples were added 10 mL of acetonitrile, and 4 g of magnesium sulfate ($MgSO_4$)/ 2 g of sodium chloride. The mixture was shaken for 1 minute. These samples were centrifuged for 5 minutes at 5000 rpm. A 2 mL aliquot of the upper layer was transferred to a dispersive solid phase extraction (dSPE) tube containing 150 mg $MgSO_4$, 50 mg primary secondary amine (PSA) and 50 mg ChloroFiltr®. The tubes were shaken for 30 seconds, and then centrifuged for 5 minutes at 10,000 rpm. A 300 µL aliquot was transferred to an autosampler vial and mixed with 300 µL of DI water and filtered before analyzing by LC-MS/MS. Matrix matched standards were made by spiking varying amounts (20- 4000 ng) of target pesticides to the tea extracts (external calibration).

Details are presented for the liquid chromatography performed in gradient mode using **A**: DI water containing 0.3% ammonium formate and 0.1% formic acid; and **B**: methanol with 0.1% formic acid using a Thermo Accucore aQ LC column (100 x 2.1 mm, 2.6 µm) and tandem mass spectrometry with ESI+.

Results: This method was found to be linear from 10-2000 ng/g, (r^2>0.995) with a LOD/ LOQ of 5 ng/g and 10 ng/g. Recoveries of the pesticides ranged from 72.5%- 149.2%. The majority of pesticides lay within 70-120%. %CV for the analysis was less than 20%. This data was achieved by an external calibration procedure. This newly developed method was applied to 10 real marijuana samples released to UCT.

Conclusion: Pesticide residues were detected in all of the marijuana samples tested. Methamidophos, carbendazim, acetachlor, DEET, and chlorpyrifos were among the most often detected pesticides in this study. Since there are currently no pesticide threshold values established for marijuana samples, the detected pesticide levels were compared to the European Union maximum residue levels for foodstuff (tea) samples (10 ng/ g to 5000 ng/g). Some pesticides were found exceeded these levels, with a few at extremely high concentrations, such as 67070 ng/g carbendazim found in sample #8, and 75137 ng/g chlorpyrifos in sample #7. The findings indicated that pesticides were used for higher marijuana production, thus medical marijuana should be inspected for pesticide residues before the use by patients who suffer from HIV, AIDS, and cancers.

Keywords: Pesticide Residues, Marijuana Contaminants

P113
Blood Alcohol Elimination Rates Among Miami-Dade DUI Arrestees from 2009-2013

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Introduction: Alcohol metabolism rates can be affected by factors including individual metabolic function, genetic and phenotypic factors, and food ingestion. Questions may arise when an individual is arrested for driving under the influence if there is a time internal between collection of the blood sample and time of incident.

Objective: Alcohol elimination has been studied, however usually in a controlled laboratory environment. Road-side breath ethanol results are often not permissible in court; therefore, attorneys rely on blood ethanol content of ethanol obtained by headspace gas chromatography (HS/GC).

Method: Individuals arrested in Miami-Dade County for suspected DUI or individuals involved in major traffic crashes are required to submit whole blood for ethanol analysis. These samples are delivered to the University of Miami toxicology lab for alcohol testing. Individuals with two separate blood collections occurring sometime after an initial traffic stop or crash were included. Subjects' ages ranged from 19-65 years, two of which are unknown. The sample pool includes both male and female from various ethnic backgrounds. Ethanol quantitation was determined by Head Space Gas Chromatography (HS/GC) dual column analysis. This method utilizes 1N-propanol as an internal standard with a 6 point calibration (0.025 – 0.3 g/100mL) demonstrating linearity of $R^2 \geq 0.999$, alongside negative, low and high controls. Sample preparation comprised of 0.1 mL of blood & 1 mL 0.15 % saline IS which then equilibrates at 55°C for 11 minutes prior to sampling.

Results: In total, blood from 62 individuals with two time points were analyzed (time intervals from 30-907 min). Data was normalized to g/100mL/hr and followed a normal distribution. The average and median ethanol decrease was 0.0182 g/100mL/hr, with values ranging from 0.009 to 0.047 g/100mL/hr^{-1}. Two values demonstrated elimination rates above 0.04g/100mL/hr. Two subjects that were excluded showed no difference in alcohol concentration between the two samples collected (t=60 min & 80 min).

Conclusion: Interpretation from our study is limited by the unknown time interval between the first sample collection and the incident. Also, we are limited by not knowing if the sampling was done in a plateau period. Most cases evaluated showed a sample collection interval > 1hr, thereby representing elimination rates over time. The two subjects that showed no difference in alcohol concentration between the two samples collected illustrate that in some instances alcohol metabolism can be equivalent to absorption and therefore elimination is indistinguishable. As expected in the remaining sample pool, the amount of ethanol in the blood from the second sample was significantly less than the initial sample collected in each subject. This is with the assumption of linear metabolism. Inter-subject variability in genetic and physiologic factors can also be contributing factors to variability seen in metabolic rates of alcohol.

Keywords: Blood Alcohol, Elimination Rates, HS/GC

P114

Simultaneous Detection and Quantification of 15 Drugs of Abuse in Whole Blood by On-Line Solid-Phase Extraction and LC-MS/MS

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Introduction: In case of requests by the Police and criminal justice system in France, the screening and quantification of drugs of abuse in whole blood is done in order to investigate driving of a vehicle under influence. A new method for quantification of drugs of abuse in whole blood was developed with a simple sample pre-treatment, online solid-phase extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS). 15 drugs of abuse and metabolites (amphetamine, metamphetamine, MDMA, MDA, MDEA, MBDB, mephedrone, 6-MAM, morphine, codeine, dihydrocodeine, ethylmorphine, cocaine, benzoylecgonine and cocaethylene) were measured in a single chromatographic run.

Objective: Develop a new drug of abuse testing by LC-MS/MS since whole blood is the specimen analyzed.

Method: For quantitative determination, 14 deuterated analogues were used as internal standards. After protein precipitation of 250 µL whole blood with ZnSO4-methanol spiked with a mixture of internal standards, samples were mixed and centrifuged; the supernatant was evaporated to dryness, and reconstituted with mobile phase, before injection in the chromatographic system. First, analytes were loaded on the extraction column (Strata-X, Phenomenex) with a 2 mM ammonium formate buffer. Then, switching the valve triggered back-flush elution on the extraction column and diverted the flow onto the analytical column (Kinetex PFP, Phenomenex) kept at 60°C. The compounds were well separated in a total run time of 15 min. A gradient was performed with the aqueous phase A (2 mM ammonium formate and 0.2% formic acid), and the organic phase B (2 mM ammonium formate with methanol-acetonitrile (70-30) and 0.2% formic acid). The mass spectrometer was an API 3200 QTrap® (AB Sciex). The method was developed in scheduled multiple reaction monitoring mode (sMRM), with two transitions per compound. All analytes were detected in positive ionisation mode.

Results: The method validation showed a recovery of the precipitation step over 75%. No loss of analytes was observed by online SPE. No matrix effect was detected by post-column infusion and by analysis of six blood samples spiked with three different concentrations (relative standard deviation < 15%). Intra-day and inter-day precisions, expressed as relative standard deviation, were lower than 15%. Accuracy was under ±15%. Calibration curves were performed from 10 to 200 µg/L for most analytes with a LOQ of 5.0 µg/L, and from 2.5 to 50 µg/L for 6-MAM and cocaine with a LOQ of 1.25 µg/L. The method was linear with $r > 0.995$. The linearity was verified up to 1000 µg/L and 250 µg/L, respectively for each calibration range. Results were compared with the liquid-liquid extraction-gas chromatography-mass spectrometry (GC-MS) method, with a good correlation.

Conclusion: This method showed satisfactory sensitivity, accuracy, precision and linearity. Benefits were multiple: low sample volume, highly reduced time of sample preparation, short chromatography and simultaneous analysis of basic drugs of abuse measured in forensic toxicology, as well as a cathinone compound. The present method allows measuring simultaneously amphetaminic, cocainic, opiates and mephedrone in whole blood, using on-line extraction and LC-MS/MS. It was completely validated and successfully used in routine in driving under the influence of drugs cases and forensic analysis.

Keywords: On-Line Solid-Phase Extraction, LC-MS/MS

P115
Identification and Quantitation of Designer Drugs in Urine by LC-MS/MS

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Introduction: Recently, trends in the drugs of abuse field suggest novel compounds, similar in structure to current drugs of abuse, are rapidly appearing on the market in an attempt to evade controlled substance laws. These "designer drugs" or "legal highs" have caused concern due to their unknown quantity in terms of potency, side effects, health consequences and potential for abuse.

Objective: A method is presented here using a simple dilute and shoot sample preparation, using the QTRAP 3200 to quantify and identify a number of these compounds in urine, specifically ketamine, norketamine, dehydronorketamine, 3-methoxyeticyclidine, 3-methoxyphencyclidine and methoxetamine, generating library searchable spectra for high confidence identification.

Method: The sample preparation consists of a simple pre-treatment step using dilution of urine samples (1:5 in aqueous mobile phase), followed by direct injection at a volume of 20ul. HPLC methodology consists of a water/methanol/buffer mobile phase with a short gradient on a Kinetex 50x2.1 PFP column. Data was generated using a Multiple Reaction Monitoring (MRM) method with two transitions per compound and an MRM triggered Enhanced Product Ion (EPI) method to not only obtain quantitative results but additionally allow confirmation of the analytes using library searchable MS/MS spectra from the same injection.

Results: Using this method it was possible to analyse the compounds ketamine, norketamine, dehydronorketamine, 3-methoxyeticyclidine, 3-methoxyphencyclidine and methoxetamine with an injection - injection time of 8 minutes. Sensitivity for the compounds is shown to be between 1 and 5ng/ml in urine. This equates to 1 to 5pg on column sensitivity for these compounds.

Quantitative performance has been demonstrated and shows accuracies within 20 % of nominal at the LOQ and %CV of 20 %, also at the LOQ and within 15 % for the higher concentrations.

Mean linearity (r2) has been shown for all compounds to be > 0.993 across at least three orders of magnitude. Additional verification parameters including matrix effects have been investigated.

Conclusion: An LC-MS/MS method for the analysis of 6 designer drugs was achieved utilizing a designer drug library for high confidence identification.

Keywords: QTRAP 3200, Designer Drug Identification

P116

Comprehensive Accurate Mass MS/MS Drug Screening Using a Q-TOF Instrument and New Software Tools in a Simultaneous Targeted and Non-Targeted Approach for Urine Samples

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Introduction: For Research Use Only. Not For Use in Diagnostic Procedures. Laboratories performing drug screening analyses on samples often wish to identify as many compounds as possible from a single experiment. As a general rule, the complexity of the analysis and the data interpretation increases with the number of target compounds, thus increasing the workload for technologists and data analysts. Furthermore, not only do analysts want to identify compounds from a target list of known drug agents, they are also interested in finding out what other potential drugs or compounds might be in their samples that they don't know about. A complete solution has been elusive, requiring either a duplicate injection or at least multiple passes of data processing.

Objective: In this study we aim to show a comprehensive targeted and non-targeted sample analysis that further allows retrospective analysis without re-acquiring the data.

Method: A standard set of urine toxicology samples (50) was analyzed for a large panel of drugs of interest (1250 separate drugs). The samples were diluted and then analyzed to reduce background. A general method was developed for use on a UPLC Q-TOF system. The Q-TOF not only generates high resolution accurate mass data, it also captures full scan MS/MS data that can be used to search against available compound databases to increase the confidence level of a potential identification. This method employs a non-targeted data acquisition approach in which a TOF-MS survey scan was used to trigger up to 20 information-dependent scans per cycle for all analytes detected. Method parameters:

- **Sample Preparation:** Samples in urine matrix were prepared using a simple "dilute and shoot" approach. The diluent was mobile phase A, and a dilution factor of 20x was used.
- **HPLC Conditions:** A Shimadzu Prominence LC system with a Phenomenex Kinetex C18, 50x2.1mm, 2.6um column at 30°C with a generic chromatographic gradient of eluent A (water + 2mM ammonium formate + 0.1% formic acid) and eluent B (acetonitrile + 2mM ammonium formate + 0.1% formic acid) was used at a flow rate of 500µL/min. The injection volume was set to 10µL.
- **MS/MS Conditions:** An AB SCIEX TripleTOF™ 4600 LC/MS/MS system with Turbo V™ source and Electrospray Ionization (ESI) probe was used (positive mode, curtain gas 30, gas 1 60, gas 2 60, spray current 5000, temperature 400°C).

Results: The data was analyzed using the new a new software package, MasterView, that allows a simultaneous targeted (XIC list based) and non-targeted search algorithm. Spectra generated in these experiments are searched against a library of over 1200 compounds and linked to an automated ChemSpider session to help identify non-targeted compounds in the samples. Formula finder and spectral fit tools are also available to help elucidate unknown compounds in the samples. The new software results were compared to XIC Manager in terms of ease of use and speed.

Conclusion: Results of this study show the effectiveness of a comprehensive workflow using TripleTOF® technology coupled with powerful data analysis software

Keywords: Comprehensive Analysis of Targeted and Non-Targeted Compounds

P117

A Case of 4-bromo-2,5-dimethoxy-N-[(2-methoxyphenyl)methyl]-Benzeneethanamine (25B-NBOMe) Intoxication: A New Potent 5-HT2A Agonist Designer Drug

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Introduction: 4-bromo-2,5-dimethoxy-N-[(2-methoxyphenyl)methyl]-benzeneethanamine (25B-NBOMe) is a N-benzyl phenethylamines derivative. *In vitro* binding studies have demonstrated that this compound is a potent serotonin 2A (5-HT2A) receptor agonist. The 5-HT2A receptor has been closely linked to complex behaviors including working memory, cognitive processes and affective disorders such as schizophrenia. These receptors are believed to mediate the primary effects of hallucinogenic drugs. Little to no pharmacokinetic or pharmacological data concerning man or animals is presently available in the literature. Nor is there a published method for the determination of 25B-NBOMe in biological specimens.

Objective: We present a case of 25B-NBOMe intoxication and a method for detection and quantification in specimens from this case.

Case History: A 19 year-old male was taken to the emergency department (ED) in April of 2013 in status epilepticus with a rectal temperature of 104ºF. His roommates had called Emergency Medical Services after finding him unresponsive with generalized grand mal seizure activity. He was placed on a propofol infusion and given pancuronium to control his agitation. Seizure-like activity ceased with these medications. Other signs and symptoms included: diaphoresis, facial cyanosis, dilated pupils unresponsive to light, sinus tachycardia and respiratory distress. During hospitalization, the patient required continuous treatment to control seizures for three days, and was sedated due to severe agitation for an additional three days. On the second day of hospitalization, a friend admitted that the patient used "some unknown drug, called 25B." Serum and urine collected at the time of admission were sent to our laboratory for analysis.

Method: A high pressure liquid chromatography with electrospray ionization triple quadrupole mass spectrometry (HPLC/MS/MS) method for the identification and quantification of 25B-NBOMe using 2-(2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine (25H-NBOMe) as the internal standard (ISTD) was developed using a liquid/ liquid extraction. The chromatographic separation was performed on a Luna C8 (2)100A 100x2.0 mm, 3-µ column. Detection was accomplished by multiple-reaction monitoring (MRM) via electrospray ionization (ESI) source operating in the positive ionization mode.

Results: Calibration curves were linear over the investigated concentration range, 25 to 2000 pg/mL, with a lower limit of quantification (LOQ) of 25pg/mL. The accuracy/bias of the assay at four different QC concentrations varied from a low of 89% at a concentration of 300 pg/mL to 112% at a concentration of 1500 pg/mL. The inter-day precision at the four different QC concentrations ranged between -18% and 12%, and the intra-day precision ranged between -14% to 4%. The patient's 25B-NBOMe serum and urine concentrations were 180pg/mL and 1900pg/mL, respectively. No interferences were observed from compounds in commercially available TDM and drugs of abuse controls.

Conclusion: The presented method may be used to reliably detect and quantify 25B-NBOMe in serum.

Keywords: 25B-NBOMe, HPLC/MS/MS, Designer Drugs

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P118

A High Performance Liquid Chromatography Tandem Mass Spectrometry Method for the Determination of JWH-250, CP47,947 and its C8 Homologue in Whole Blood

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Introduction: Synthetic cannabinoid compounds have been identified as the active intoxicants in products marketed as "herbal incense products" (HIPs). Although these products claim, "not for human consumption", they have become popular as a legal high. This is due to their psychoactive properties that cause effects similar to those achieved by smoking marijuana. The cyclohexylphenol derivative, CP47,497 and its C8-homologue were among the most commonly encountered active ingredients found in HIPs prior to their scheduling by the DEA. JWH-250 is still found in HIPs today.

Objective: To develop a method for the quantification of CP47,497, its C8-homologue and JWH-250 in mouse whole blood as part of dose response, disposition, abuse potential and behavioral studies concerning these cannabimimetics.

Method: We developed a high pressure liquid chromatography tandem mass spectrometry (HPLC/MS/MS) method for the detection and quantification of CP47,497, its C8-homologue and JWH-250 in whole blood. Following the addition of deuterated internal standards, the cannabimimetics were isolated by liquid-liquid extraction with cold acetonitrile. Chromatographic separation was performed on a Zorbax Eclipse XDB-C18 column, 4.6 x 75 mm, 3.5mm (Agilent Technologies, USA). The mobile phase consisted of a 20:80 v/v solution of water-acetonitrile with 0.1 mM ammonium formate. An injection volume of 10 µL was used with a mobile phase flow rate of 0.5 mL/min and a total run tine of 12 minutes. The following transition ions (m/z) were monitored for CP47,497; 317>299 and 317>245; its C8-homologue; 331>259 and 331>313 and JWH-250; 336>121 and 336>91. The method was evaluated for absolute recovery, ion suppression, accuracy/bias, inter-day and inter-day precision, bench top stability, freeze/thaw and post-preparative stability.

Results: Duplicate calibration curves were determined to be within 20% of the nominal value for each analyte. The linear regression correlation coefficients for each analyte's calibration ranged from $r^2 = 0.995$ to 0.998. The calibration of CP47,497 and its C8-homologue were linear from 5 to 200 ng/mL, while JWH-250 was linear from 1 to 200 ng/mL. The lower limit of quantification (LOQ) and the lower limit of detection (LOD) were administratively set for CP47,497 and C-8 homolog at 5 ng/mL and 2.5 ng/mL. The LOD and LOQ were administratively set for JWH-250 at 1.0 ng/mL. Assay performance was evaluated using a set of four quality control specimens. Accuracy/bias of the assay was determined to be within +/- 20% of the target value for each analyte at each quality control value. Intra-day and inter-day precision samples were determined not to exceed a 15% CV except for the LOQ which did not exceed a 20% CV.

Conclusion: The method has been found acceptable for the quantification of CP47,497, its C8-Homologue and JWH-250 in mouse whole blood.

Keywords: Quantification of CP47,497, Synthetic Cannabinoid

This project was supported by the National Institute on Drug Abuse (NIDA) Center for Drug Abuse grant P50DA005274.

P119

Validation and Implementation of a Randox Laboratories Evidence Analyzer Enabling the Establishment of a New Case Analysis Scheme

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Introduction: The Virginia Department of Forensic Science analyzes biological specimens for the presence or absence of drugs and alcohol in DUI/DUID, medical examiner, and police cases. A comprehensive screening panel is essential to the laboratory due to the broad scope of drug testing. A qualitative screening method has been validated using the Evidence Analyzer from Randox Laboratories, Ltd. The Evidence Analyzer validation enabled the implementation of a defined scope of testing for DUID cases as well as a more comprehensive immunoassay-based screen for medical examiner and police cases facilitating an improved case workflow.

Objective: To validate one commercially available and one custom biochip containing thirty discrete testing regions using a competitive immunoassays to enable the implementation of an expanded DUID, medical examiner, and police case screening panel. The assays included in the validation are: opiates, benzodiazepines, barbiturates, PCP, amphetamine, methamphetamine/phentermine, MDMA, buprenorphine, cannabinoids, cocaine/cocaine metabolite, methadone, tricyclic antidepressants, carisoprodol/meprobamate, citalopram, tramadol, trazodone, zaleplon, zolpidem, zopiclone, acetaminophen, dextromethorphan, fentanyl, ketamine/norketamine, LSD, mephedrone, salicylate, and meperidine.

Method: A thorough validation was conducted for each assay on the Evidence Analyzer biochip. The validation consisted of an evaluation of limit of detection, cut off level precision, assay cross reactivity, stability, sample preparation, urinalysis, and previously analyzed case samples. Samples were prepared by spiking blank blood with the appropriate analytes. The spiked samples were then diluted using a 1/4 ratio with manufacturer supplied diluent prior to analysis.

Results: The cut off was established and validated for all assays which met the predetermined acceptance criterion of a coefficient of variance no greater than ±20 %. The cut off was further evaluated by assessing concentrations ±50 % of the cut off. A two standard deviation range was established for each concentration and evaluated. The precision at the cut off was assessed with triplicate analysis over five days. Upon establishment of the cut off, the cross reactivity for other analytes was determined. To determine the cross reactivity, the measured concentration of the analyte was compared to the measured concentration of the target. The instrument is considered semi-quantitative and utilizes a nine point calibration curve to establish concentrations. The stability of the calibration was established by evaluating multiple calibrations. Throughout the validation, the calibration curves were stable and in close relation to each other.

Conclusion: The validation of the thirty assays on the Randox Evidence Analyzer was successful. The implementation of the new screening assay enabled restructuring of analysis schemes for DUID cases. Previously, a three tiered system was utilized that included two screening techniques. The new analysis scheme involves a more comprehensive screening using the Evidence Analyzer as well as a more defined scope of testing for DUID cases. The Evidence Analyzer also enables for a more comprehensive immunoassay-based screen for medical examiner and police cases. A secondary screen is still available for these cases when deemed necessary. Overall, the Evidence Analyzer is a fully automated system with a drastically reduced analysis time in comparison to the previously utilized semi-automated system while expanding the screening capabilities.

Keywords: Immunoassay, Randox, Screening Techniques, Qualitative Method Validation

P120

Broad Toxicological Screening Results From Suspected GHB/GBL Overdose Cases

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Introduction: Gamma-hydroxybutyrate (GHB) and gamma-butyrolactone (GBL) use can result in significant acute toxicity. To date there is limited data on the drugs that are co-ingested in individuals presenting with GHB/GBL toxicity.

Objective: The aim of this study (which was part of wider, approved study to evaluate the utility of dried blood spots as an alternative to venous blood for GHB analysis) was to assess the drugs present in a cohort of patients with a clinical diagnosis of acute GHB/GBL toxicity.

Method: Patients included in this study were from those admitted to the Emergency Department of an inner-city London teaching hospital with a clinical diagnosis of acute GHB/GBL toxicity. Anonymised plasma samples from consenting patients were prepared by liquid:liquid extraction and analysed using UPLC in combination with time-of-flight (TOF) mass spectrometry. Chromatographic separation was achieved using a Waters ACQUITY UPLC® I-Class system fitted with a HSS C_{18} column maintained at 50°C and eluted with a mixture of ammonium formate at pH 3 and acetonitrile containing 0.1% formic acid. The total chromatographic run time was 15 minutes. Data were acquired using a Waters Xevo G2S QTOF in MS^E mode which involves the rapid alternation between two collision energy regimes. Data is recorded in two functions: the first, acquired at low energy, provides the accurate mass of the precursor ion; the second, at elevated energy (ramp 10-40eV), provides the accurate mass of the fragment ions for additional confirmatory purposes. Data were processed using the UNIFI™ Toxicology Screening Solution (Waters) and compared to a comprehensive database, prepared under the same conditions, containing more than 1000 drugs and metabolites. Identification of substances by UPLC-TOF-MS^E was based on a combination of retention time and a mass 'fingerprint' for each analyte, the latter comprising the accurate mass of the precursor ion and fragment ions.

Results: Samples were available for analysis from 15 patients. All samples had screened positive for GHB by a separate analysis performed at an independent laboratory. Three samples were negative for additional recreational drugs, as determined by UPLC-TOF-MS^E. All other samples were positive for mephedrone (4-methylmethcathinone; 4-MMC) and its metabolites. Three of the samples contained mephedrone as the only recreational drug. Two of the samples were also positive for 4-methylethcathinone (4-MEC). Mephedrone was found in combination with other drugs including: ketamine and metabolites (*n=4*); methamphetamine (*n=5*); amphetamine (*n=1*) and benzodiazepines (*n=1*). Other detected drugs included: antiretroviral drugs, common antibiotics and over-the-counter medications such as anti-histamines, paracetamol.

Conclusion: UPLC-TOF-MS^E was used for its ability to screen for both established recreational drugs (targeted analysis) in addition to novel psychoactive substances (non-targeted analysis). In this study a high prevalence of mephedrone was noted in a cohort of patients with a clinical diagnosis of acute GHB/GBL toxicity. The majority of the mephedrone-containing samples were also positive for other recreational drugs such as methamphetamine and ketamine. Studies such as this, with broad-based, toxicology screening, are important to determine the actual drugs used by individuals presenting to hospital with acute recreational drug toxicity and to determine trends in the use of, and toxicity associated with, novel psychoactive substances.

Keywords: GHB, Recreational Drugs, Mephedrone, Toxicological Screening, UPLC-TOF-MS^E

P121

Highly Automated SPE / MSMS Analysis of Gabapentin and Pregabalin in Urine

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Introduction: Gabapentin (Neurontin™) and Pregabalin (Lyrica™) are medications used to treat chronic pain and are often a part of urine drug testing protocols.monitoring safety and compliance. These drugs are passed into the urine intact with very little metabolic effect on structure. Thus, these drugs can be present at very high levels, often in excess of 100,000 ng/mL. Analytical methods for these drugs include GC/MS and UPLC/MS/MS.

Objective: To explore the use of a highly automated Solid Phase Extraction (SPE) / MS/MS technique in the simultaneous analysis of Gabapentin and Pregabalin.

Method: Urine samples submitted from patients receiving Gabapentin or Pregabalin were diluted 50x with a Gabapentin D10 internal standard (IS) in water using a Tecan EVO 2000 sample handler. The Tecan accurately adds 12 µL of sample to 588 µL of water/IS in a 96 deep well plate to accomplish the dilution. Analysis was then completed using an Agilent RapidFire™ (RF) coupled to an Agilent MSMS (6460) using an ESI source. Samples were loaded onto an SPE micro cartridge in the RF system with a 3 sec segment and eluted using a 7 sec segment. Carryover at high concentrations resulted in the need for a blank between each sample, such that the net time per sample on instrument was 25 sec. Linearity was established using calibrators prepared in normal human urine and diluted as described above. Positive and negative controls were run with each batch of patient samples.

Results: The RF/MSMS method demonstrated excellent linearity (as evidenced by R^2 values greater than 0.99) and sensitivity with LOD/LOQ of 1000ng/mL for both Gabapentin and Pregabalin. The ULOL of 500,000 ng/mL is consistent with sample values seen in this population. As an example of method robustness, over 20,000 injections (10,000 samples and 10,000 blanks) were completed without issue on the same micro SPE cartridge making the putative cost per sample substantially less than with traditional analysis. Matrix and possible interferences were insignificant (matrix effect less than 10%) as would be expected from an SPE method. Precision and accuracy were acceptable (within 25% deviation from target and less than 15% coefficient of variation) over 3 days at 3 concentration levels.

Conclusion: Comparison of patient data from RF/MSMS with data obtained using a conventional UPLC/MS/MS (2 min run time) demonstrated the excellent fidelity of the data. Even with a blank between samples, this method is at least 4x faster than the corresponding UPLC/MS/MS method. It is about 7 times less expensive to run in terms of maximum injection count per SPE cartridge versus LC column, without consideration of solvent use.

Keywords: SPE/MS/MS, Gabapentin, Pregabalin

P122

Extraction of Synthetic Cannabinoids from Urine Using Supported Liquid Extraction (SLE) prior to GC/MS Analysis

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Introduction: Synthetic cannabinoids are compounds which allegedly mimic the effects of Δ^9-THC found in nature by interacting with the CB_1 and CB_2 receptors in the brain. Since 2004 they have been obtainable via herbal incense blends from head shops and the internet, and as the popularity of these compounds has increased, so too has the awareness of their dangers. As governments attempt to legislate against these synthetic compounds, (in the United States, JWH-018 was outlawed in 2009), the "internet" responds by filling the gap with newer analogs. This trend inevitably creates a need for specific, sensitive and fast assays in forensic toxicology labs that look at parent and metabolite compounds excreted in urine following drug consumption.

Objective: The objective was to develop a GC/MS assay for the determination of synthetic cannabinoids from hydrolyzed using Biotage ISOLUTE SLE+ (supported liquid extraction). The SLE extraction mechanism is very efficient, delivering higher analyte recoveries and cleaner extracts than equivalent LLE methods.

Method: Blank human urine was spiked with a synthetic cannabinoid panel consisting of UR144, JWH073, JWH018, 5-hydroxypentyl-JWH250, 3-hydroxybutyl-JWH073, AM2201, 4-hydroxypentyl-JWH018, 5-hydroxypentyl-JWH018 and JWH200.

Extraction conditions were evaluated using spiked urine, pre-treated 1:1 (v/v) with 100 mM ammonium acetate pH 5. Samples were hydrolyzed using 50 µL (approx. 4500U) ß-glucuronidase for 2 hours in a water bath at 60°C. Sample preparation was performed on 1 mL SLE+ columns using 1 mL of pre-treated urine and on 400 µL SLE+ columns using 400 µL of pre-treated urine. Extraction on the 1 mL format was performed using 2 x 3 mL volumes of 90:10 hexane:ethyl acetate and 2 x 1 mL volumes of 90:10 hexane:ethyl acetate on the 400 µL format. Samples were evaporated with air and derivatized with 25 µL ethyl acetate and 25 µL 99:1 BSTFA:TMCS. The samples were transferred to glass vials, sealed with non-split caps and heated to 70°C for 30 minutes prior to GC/MS analysis. All samples were analyzed using an Agilent 7890 GC with a 5975 MSD. Chromatography was performed on a BPX-5 SGE capillary column; 30 m x 0.25 mm ID x 0.25 µm using 1.2 mL/min helium as the carrier. Positive ions were acquired using electron ionization operated in SIM mode.

Results: Analyte peak areas from samples that were spiked before and after extraction were compared to determine percentage recovery when using the SLE procedure. Robustness of the extraction method was assessed using volunteer urine from 4 sources and gave close agreement. Recovery profiles were determined to be 83%-112% for all analytes in 4 urine donors and RSDs were below 10%. For method development purposes the initial spike concentration used was 200 ng/mL when extracting 0.5 mL matrix. Linearity was acceptable, demonstrating coefficients of determination greater than 0.99 over concentration levels 5 -500 ng/mL.

Using 1 mL of sample (0.5 mL urine) the limits of quantitation were: 5-hydroxypentyl-JWH250: 5 ng/mL, JWH200: 30 ng/mL and the remainder analytes: UR144, JWH073, JWH018, 3-hydroxybutyl-JWH073, AM2201, 4-hydroxypentyl-JWH018, and 5-hydroxypentyl-JWH018 were 15 ng/mL. In order to increase the sensitivity, the reconstitution solvent volume could be evaluated.

Conclusion: This poster provides a quick, simple and reliable protocol for the extraction of synthetic cannabinoids from hydrolyzed urine prior to GC/MS, demonstrating high, reproducible extraction efficiencies and acceptable limits of quantitation from multiple urine donors. This fulfils what was set in the objective stage.

Keywords: Supported Liquid Extraction, Synthetic Cannabinoids, Urine, GC/MS

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A Novel Fully Automated Process for the Hydrolysis and Supported Liquid Extraction of Basic Drugs Using Tecan Freedom EVO 100 Prior to HPLC-MS/MS Analysis

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Introduction: Sample preparation methodology that extracts and concentrates an analyte prior to analytical analysis is typically necessary to facilitate accurate quantitative and qualitative results. A fully automated high throughput sample preparation protocol would be extremely useful to address the high volume of drug testing conducted every year.

Objective: Here we describe a novel automation process where basic drugs are hydrolyzed and extracted from urine samples using a Tecan Freedom EVO workstation in combination with the Biotage Supported Liquid Extraction (SLE+) technology in a 96 well plate format. The workflow fully automates sample barcode identification, the enzymatic hydrolysis step, the addition of internal standard, and the extraction of the drugs using the supported liquid extraction (SLE) format. The entire process can be conducted without moving any of the sample or extraction plates making the automation set-up less costly than what would be required for an automated solid phase extraction method.

Method: A Tecan workstation was set-up for sample hydrolysis, sample pre-treatment, and subsequent sample extraction to give a continuous automated workflow. Over 70 urine samples were spiked with basic drugs (e.g. opiates and benzodiazapines) at 20ng/mL concentration and processed using the fully automated workflow. The sample preparation method was fully automated by building individual workstations dedicated to each step of the process on a Tecan liquid handling platform. A series of calibration standards were processed to generate calibration curves for the analytes. Mock patient samples were prepared by spiking blank urine with different amounts of the basic drugs prior to processing using the fully automated workflow design. The mock patient samples were mixed with β-glucoronidase in a 96 well plate and the solutions agitated and heated to 50 °C for 30 minutes to simulate the hydrolysis step. An aliquot of the hydrolysed samples was pipetted into second 96 well plate where they were mixed with pre-treatment solution (2% ammonium hydroxide). The pre-treated samples were then loaded on a SLE+ 96 well plate and extracted using ethyl acetate. The extracted samples were dried down, reconstituted and analysed using an Applied Biosystems 4000 Q-Trap with an Agilent 1200 liquid chromatographic system.

Results: The observed averaged recoveries ranged from 50-120% with intra-run %RSDs less than 20% for the analytes of interest across 70 urine samples. The higher intra-run CV was attributed to less than optimal sample mixing and transfer using automated system. This could be remedied by further programing of Tecan system to optimize these steps. Most of the drug concentrations in the mock patient samples were calculated to within ±15% of the know values using the generated calibration curves.

	Patient Sample Number: Calculated Drug Concentration in ng/ml (%Accuracy)								
Drug Analyte	#2	#7	#8	#9	#12	#14	#16	#21	#27
Alprazolam	**64.1 (86)**			**71 (84)**			**43 (81)**		
Clonazapam	55 (100)	29.6 (99)		62.5 (105)		54.2 (118)			59 (107)
Diazepam			**30 (118)**		**57.7 (114)**				**61.5 (110)**
Flunitrazepam		25 (84)				52.6 (117)		41.9 (105)	
Oxazepam	**58.9 (93)**						**36 (97.9)**		**64.8 (84.8)**
Oxycodone	68 (81)	37.3 (81)							
Hydrocodone	**63.5 (87)**	**32.7 (92)**					**42.5 (83)**		
Fentanyl			32.7 (78)				41.8 (84)		54.7 (100)
Buprenorphine	**50.4 (107)**		**28.9 (86)**	**63.9 (94)**	**51.6 (97)**			**51.2 (80)**	**53.9 (101)**
EDDP	53.6 (98)				45.5 (90)		36 (101)	35 (87)	

Conclusion: We present a fully automated approach for the hydrolysis and extraction of multiple drugs with good recoveries and quantitative performance in a 96 well format.

Keywords: Automation, Supported Liquid Extraction, Multi-Drug Screening, Sample Preparation

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Cation Exchange Solid Phase Extraction Gets a Make Over: A New Abbreviated Cation Exchange SPE Method with No Dry Down Towards the Extraction of Basic Drugs from Urine using EVOLUTE® Express CX Prior to HPLC-MS/MS Analysis

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Introduction: The ability to quickly conduct routine drug analysis methods is highly regarded by drug testing laboratories as a necessary capability that helps to improve day to day workflow and efficiency.

The high demand for routine testing for schedule I and pharmaceutical drug abuse in the workplace and as mandated by law further reinforces the need for fast multi-drug testing capabilities.

Objective: Here, we demonstrate a new cation-exchange solid phase extraction sample preparation method for basic drugs using recently developed 96 well plate design technology and a novel extraction solvent that eliminates the dry down process.

Method: Blank human urine was spiked with a multi-suite of basic drugs. The urine samples (200 µL) were pre-treated with 0.01N HCL (200 µL) and loaded onto EVOLUTE Express CX 96 well plate. The new plate design technology allows for samples to be loaded directly onto the sorbent with no conditioning or equilibration steps needed, thus shortening the workflow process. This abbreviated processed is referred to as a "Load-Wash-Elute" strategy. The analytes were extracted with a novel organic solvent mixture of tetrahydrofuran, methanol, acetonitrile, and ammonium hydroxide that facilitates the recovery of the targets with high efficiency and allows for samples to simply be further diluted with water and injected directly onto HPLC column prior to analysis by positive electrospray ionization in MRM mode.

Results: The retention of the target analytes onto a resin cation exchange sorbent was observed with good efficiency in the absence of conditioning and equilibration of the sorbent prior to loading. The resin was washed of ionic and neutral interferences using water and aqueous acidic isopropanol solutions to reduce ion suppression effects during LC-MS/MS analysis. Ion suppression was observed to be less than 25% for all analytes. The analytes were extracted using a minimal amount of the novel extraction solvent (600µL) to yield recoveries >75% with %RSDs less than 10%.

Conclusion: We present a new abbreviated cation exchange method for the extraction of multiple basic drugs and subsequent analysis with no dry down steps required.

Keywords: Multi-Drug Screening, Sample Preparation

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Resources for Responding to the Challenge of Emerging Drugs… Where is the Information Your Analysts Need?

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Introduction: Forensic laboratories are faced with a challenge when attempting to identify unknown compounds in a sample substance. The rise in prevalence of new designer drugs known as "Spice" and "Bath Salts" has increased this challenge as uncontrolled drug analogs rapidly appear on the drug market. There exists a need in the community to provide laboratory personnel with assistance in identifying these emerging designer drugs as many of these compounds have yet to be identified and characterized in the literature.

Objective: The objective of this presentation is to provide the community with an overview of the growing array of information and resources available to aid in identification and interpretation of data related to emerging drugs.

Method: In October 2012 NIJ sponsored an analog working group meeting at which major players from across several disciplines of forensic science were represented, including commercial vendors, research institutes, federal, state, and local crime laboratories, DEA, and customs. Input on the most valuable resources utilized by these attendees was recorded and compiled for distribution. Additional resources identified by RTI scientists are also included.

Results: Resources include downloadable EI-MS spectral libraries, both commercial and freely available, as well as Web accessible databases of multiple spectral methods. Other resources include drug monographs, peer reviewed spectral data, and active discussion forums.

Conclusion: Several resources are available, the challenge remains to adequately disseminate this information to the forensics community. Consolidation of efforts would assist in providing the information in multiple formats to be useful to the widest possible audience.

Keywords: Forensic Resources, Spectral Databases, Designer Drugs

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Development and Application of ELISA for Detection of Synthetic Cannabinoids: JWH-018, JWH-073, JWH-200, JWH-022, AM-2201, AM-2232, AM-1220, UR-144 and XLR-11 in Oral Fluid

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Introduction: The last 2-3 years have seen the emergence of numerous indole-derived synthetic cannabinoids. They mimic the behavior of THC, by binding the CB_1 and CB_2 receptors. The ease of chemical modification to the core indole ring, has allowed for a plethora of compounds being synthesized, which constantly keep ahead of current drug enforcement laws. By June 2012, 15 synthetic cannabinoids (viz. JWH/AM compounds) were placed in the Schedule I substances category and in April 2013, the DEA announced the intent to exercise their emergency scheduling authority to place UR-144, XLR-11 and AKB-48 into the same category.

Objective: The primary objective of this study was to develop immunoassay screening methods for detection of the parent JWH and AM type compounds, as well as the newly emerging and currently more prevalent; UR-144 and XLR-11 compounds in oral fluid.

Method: Oral fluid specimens were collected with Quantisal™ devices. The ELISA method utilizes either JWH-018 or UR-144 antibodies immobilized on microtiter plates. The calibrators (75µL; either JWH-200 or UR-144 respectively) and oral fluid specimens (75µL) were pipetted in duplicate and ***pre-incubated*** (30 min). JWH-018 or UR-144 enzyme conjugate (100µL) respectively, is added and incubated (60 min). The plates were washed with DI water, then incubated with substrate (30 min), then stopped with 1N HCl and read at 450 nm, using a plate reader. The newly developed ELISA methods were applied to 96 authentic oral fluid specimens; 46 negative and 50 previously confirmed positive for one or more synthetic cannabinoids included in this procedure, by a fully validated LC-MS/MS procedure utilizing a cut-off of 0.25ng/mL for all analyzed compounds.

Results: The ELISA cutoff concentrations were 0.25ng/mL for JWH-200 and 1ng/mL for UR-144. The assays detect the parent compounds: JWH-200, JWH-018, JWH-073, JWH-022, AM-2201, AM-2232, AM-1220, UR-144 and XLR-11. There was no cross-reactivity with other drugs of abuse/prescription medications. 32 out of the 96 specimens were analyzed with JWH-018 assay method: Pos (21); Neg (6), with 5 false negatives by ELISA, of which 4 contained <0.5 ng/mL AM-2201 and 1 contained JWH-210 that does not cross-react with the assay. The other 64 specimens were analyzed with UR-144 assay method: Pos (23); Neg (40), with 1 false negative by ELISA, containing <0.3 ng/mL XLR-11.

Conclusion: The ELISA assays employ a critical sensitivity-enhancing pre-incubation step, for detection of the parent JWH, AM, UR-144 and XLR-11 compounds in oral fluid. These methods allow toxicologists to utilize oral fluid matrix to screen for a range of synthetic cannabinoids.

Keywords: JWH-018, AM-2201, UR-144, XLR-11, K2, Oral Fluid, ELISA, LC-MS/MS

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Direct Injection LC-MS/MS Analysis of Opiates, Methamphetamine, Buprenorphine, Methadone and Their Metabolites in Oral Fluid from Substitution Therapy Patients

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Introduction: As an alternative matrix for drug testing, oral fluid is relatively cleaner and more accessible to sampling. The sampling process can be readily supervised, reducing risks of adulteration or substitution; while cleaner matrix facilitates direct LC/MS/MS analysis.

Objective: In this study, we explored the LC-MS/MS methodology, with a simple sample preparation step, for simultaneous quantification of heroin, methamphetamine, buprenorphine, methadone and their metabolites (6-acetylmorphine, morphine, codeine, amphetamine, norbuprenorphine, EDDP) in oral fluid.

Method: Clinical oral fluid specimens were collected upon patients' arrival for their daily dose. In most cases, patients' last doses were taken approximately 23 h earlier. Patients were asked to rinse prior to sample collection and the samples were stored at –20 ^{o}C until use. IRB protocols established by E-Da hospital were followed for sample collection and information processing and usage. For analysis, 40 µL of thawed oral fluid was fortified with 10 µL of 10-internal standardsolution (0.1 µg/mL). Samples were briefly vortex-mixed and centrifuged at 12000 rpm for 10 min. Ten microliter of supernatant was injected onto the LC-MS/MS system. Standard oral fluid samples were prepared similarly using oral fluid collected from laboratory personnel. Chromatographic separation was achieved using an Agilent Zorbax SB-Aq (100 mm x 2.1 mm i.d.; 3.5 µm particle) analytical column operated at 50 °C. The mobile phase consists of 0.1% formic acid (v/v) in water (A) and methanol (B). Under these conditions, all of the analytes eluted in less than 10 minutes with a total run time of 15 minutes. Mass spectrometric analysis was performed in positive-ion mode; applying multiple reaction monitoring (MRM) using optimized collision energy for each precursor ion designating each analyte of interest.

Results: The overall protocol was evaluated by: (a) applying the method to the analysis of laboratory-prepared standards; and (b) comparing analytical data of clinical samples derived from this and GC-MS methods. When applied to the analysis of oral fluid specimens fortified with 1–100 ng/mL of the 10 analytes of interest, this method achieved the following results: (1) inter-day and intra-day precisions range from 1.3 to 12.8% and 0.9 to 12.2% (percent CV), respectively; (2) method linearity (r^2), detection limit and quantitation limit for all analytes were >0.995, 0.1–1.0 ng/mL and 0.25–1.0 ng/mL (5 ng/mL for buprenorphine), respectively. Analytical data derived from LC-MS/MS and GC-MS analysis of methadone in 13 clinical samples were found compitable. Specifically, 10 out of the 13 sets of data overlap at the ±20% level, with the remaining 3 sets barely outside of overlap range.

Conclusion: Direct injection of freeze-and-thaw samples appears to generate favorable results for LC-MS/MS analysis of oral fluid samples. Peak shape of chromatograms derived from oral fluid containing high concentrations of certain drugs, such as morphine, codeine, and (to a lesser degree) 6-acetylmorephine and buprenorphine, show irregular characteristics, which can be improved by diluting the sample with the mobile phase prior to injection. With heroin exhibiting ideal LC-MS/MS chromatographic characteristics, inclusion of this compound in the analyte list could help detect abnormality of the analytical protocol and test specimens. Overall, our data indicate the LC/MS/MS method is more effective than our current GC-MS methods in the screening and analysis of the 10 analytes included in this study. We are currently conducting further studies on: (a) irregular chromatographic behavior of certain analytes and improvement methods; and (b) comparing analytical findings of all analytes derived from LC-MS/MS and GC-MS analysis.

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

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