Driving Under the Influence of Drugs in Florida: When the Law Misses the Mark

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Background/Introduction: According to Florida law, an individual is not guilty of driving under the influence of drugs (DUID) unless impairment is observed and is due to one or more controlled drugs listed in the Florida Statutes. Many prescription drugs, over-the-counter drugs, and novel psychoactive compounds that can cause significant impairment are not included in this list. Six other states within the USA including Alaska, Hawaii, Massachusetts, Minnesota, New York, and Oregon have similar or other restrictive language in their impaired driving statutes.

Objective: To study and discuss the potential disadvantages of driving under the influence of drugs laws that list specific compounds rather than using more general "any impairing substance" language.

Method: In Florida, urine samples are routinely collected when breath alcohol results are below 0.08 g/210L for DUID investigations. Blood samples are generally only collected when it is impossible or impractical to perform a breath alcohol test or if there is a serious bodily injury or death involved. Only one specimen type per case is routinely submitted. A volatile analysis was performed on all blood specimens. Drug analysis was performed on all urine specimens and all blood specimens involving a serious bodily injury or death. Blood specimens collected for misdemeanor cases were only analyzed for drugs if the ethanol concentration was below 0.1 g/dL from January of 2007 to September of 2014 and from June of 2017 to February of 2018, or was below 0.15 g/dL from September of 2014 to March of 2017.

From January of 2007 to February of 2018, 1796 urine specimens and 1344 blood specimens were analyzed for drugs for DUID cases. Blood specimens were screened using a basic extraction with scan gas chromatography mass spectrometry (GC-MS) and a 11 panel enzyme linked immunosorbent assay (ELISA) for amphetamines, barbiturates, benzodiazepines, buprenorphine (2013 to 2018), carisoprodol, cocaine/benzoylecgonine, fentanyl (2015 to 2018), methamphetamines, opiates, oxycodone/oxymorphone, and cannabinoids. Urine specimens were screened using a basic extraction with scan GC-MS and a nine panel ELISA for barbiturates, benzodiazepines, buprenorphine (2013 to 2018), carisoprodol, cocaine/benzoylecgonine, fentanyl (2015 to 2018), opiates, oxycodone/oxymorphone, and cannabinoids. Amphetamines and methamphetamine ELISA analytes were covered sufficiently by the urine basic extraction, hence the ELISA kits were not utilized for urine specimens. All positive results were confirmed with GC-MS and/or liquid chromatography tandem mass spectrometry (LC-MSMS).

Results: Over the past eleven years, 21% (212 out of 1028) of all drug positive blood specimens and 47% (711 out of 1527) of all drug positive urine specimens contained at least one non-controlled drug, often mixed with controlled drugs. The top ten non-controlled drugs excluding selective serotonin reuptake inhibitors (SSRIs) identified in both blood and urine are listed in Table I. The SSRIs were excluded as they are generally not impairing.

Conclusion/Discussion: The driving under the influence charges in many if not most of those cases with non-controlled drugs were either dropped or not filed. In many of these cases the issue of impairment was of little dispute. The focus was instead on whether or not the impairing drugs that were identified were included in the law. If the intent of DUID laws is to improve traffic safety by removing impaired drivers from the road, then more inclusive statutory language such as "any impairing drug" is more appropriate than linking the charge to a drug possession law framework or using other similarly restrictive language.

Table I. Top ten non-controlled drugs identified in blood and urine (excluding SSRIs)

Blood (n = 1028 positive cases)			*Urine (n = 1527 positive cases)*		
Analyte	# of Cases	% of Positive Cases	Analyte	# of Cases	% of Positive Cases
Diphenhydramine	56	5.4%	Diphenhydramine	181	11.9%
Zolpidem	45	4.4%	Zolpidem	107	7.0%
Tramadol	25	2.4%	(d) Methorphan	68	4.5%
(d) Methorphan	16	1.6%	Cyclobenzaprine	65	4.3%
Cyclobenzaprine	13	1.3%	Tramadol	62	4.1%
Trazodone	12	1.2%	Norquetiapine	58	3.8%
Topiramate	7	0.7%	Trazodone	56	3.7%
Lamotrigine	7	0.7%	Doxylamine	49	3.2%
Mitragynine	7	0.7%	Chlorpheniramine	38	2.5%
Chlorpheniramine	7	0.7%	Gabapentin	32	2.1%

Keywords: DUID, Non-controlled, DUID Laws

S2

Highway Huffing: a 10-year update on DFE related driving incidents in South Carolina

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Background/Introduction: 1,1 difluoroethane (DFE) is an inhalant commonly found as a propellant in aerosol cleaners and as a refrigerant (Freon-152a). In June 2008, a sudden increase of DFE in driving related cases in South Carolina was reported in a 2008 issue of *ToxTalk* (Volume 32, Issue 2). In this presentation we look at the trend of DFE in traffic incidents reported in South Carolina over the past 10 years and explore the difficulties in testing for this fluorinated hydrocarbon.

Objective: After completing this activity, attendees will be able to analyze trends in DFE related traffic incidents and determine if DFE is a suitable compound for their human performance testing protocols.

Method: Biological samples (blood, vitreous, urine) from traffic fatalities and DUIs were submitted from various law enforcement agencies to the forensic toxicology lab of the South Carolina Law Enforcement Division. Over 4,300 traffic fatalities and 12,500 DUI cases were analyzed from May 2008 through April 2018. Initial volatile testing was performed on a HS-GC-FID and a second test was performed on a HS-GC-MS. All results for DFE were qualitative only.

Results: From 2008 to present, DFE was only detected in 8 traffic fatalities. However, 1,1 difluoroethane was reported in 28 DUI cases. Our lab reported on average 4 DFE related traffic incidents per year from 2008-2013 with a high of 7 in 2011. Only one positive case was reported in the three years 2014-2016. However, DFE has made a resurgence with 7 positive findings in 2017 and 4 through April 2018.

Conclusion/Discussion: Ethanol remains the most abused and tested for compound in human performance testing. Toxicologists have made great efforts to keep up with emerging novel psychoactive substances. Despite the popularity of other drugs, inhalant use remains notable, especially in first time users of illicit drugs. Their affordability and accessibility as part of everyday items makes them an alternative to other drugs that are more highly regulated.

South Carolina, following its volatiles testing protocol for driving cases, has shown fairly consistent use of 1,1 difluoroethane in drivers over the past 10 years. The number of DFE cases peaked at 7, two times the yearly average, in 2011 and reached 6 in 2016. Even then, that only represents 0.5% of blood DUI cases and less than 0.3% of all traffic cases for those respective years. Since our lab uses HS-GC-MS to confirm all volatiles that are initially screened via HS-GC-FID, we were able to confirm DFE in cases without disrupting our work flow and with minimal additional cost. Laboratories that utilize other testing methods such as enzymatic tests or HS-GC-FID with 2 different columns may not be able to identify or confirm DFE or other inhalants.

Keywords: 1,1 difluoroethane, inhalants, HS-GC-MS

S3

Methamphetamine DUI/D Cases in Alabama: A 10-Year Retrospective Study

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Background/Introduction: The classic stimulant, methamphetamine, remains popular as a recreational, illicit drug. In Alabama, methamphetamine ranks as the fourth most prevalent drug found in driving cases when ethanol is not present. As a Schedule II drug, it is rarely prescribed and has limited medical usage due to its high potential for abuse. In 2012, only 16,000 prescriptions were filled for Desoxyn, the only remaining therapeutic form of methamphetamine, used to treat ADHD, obesity, and narcolepsy. The effect of low doses of methamphetamine on attention and wakefulness has been studied, but the idea of implementing routine use within society or for military purposes has not gained acceptance due to the known negative side effects (e.g. increased risk-taking) and addiction potential. Furthermore, the pharmacodynamics of methamphetamine abuse results in two distinct phases, the "rush" and "crash" phase. The side effects of these two phases have been shown to be inconsistent with safely operating a motor vehicle.

Objective: To investigate the prevalence of methamphetamine in DUI and traffic crash cases analyzed by the Alabama Department of Forensic Sciences and to evaluate methamphetamine blood concentrations, the demographics of these subjects, and common signs of impairment.

Method: DUI and traffic crash cases were evaluated over a 10-year period (2008-2017). All cases were screened by enzyme immunoassay using either a Tecan Freedom Evo 75 with Immunalysis reagents or a Randox Evidence Analyzer. Confirmation and quantification were performed by SPE followed by GC/MS analysis. Cases involving Drug Recognition Expert (DRE) evaluations or cases where "cognitive performance enhancement" was used as a DUI defense were isolated as case studies. Prevalence data overlaid onto a map of the State of Alabama was created to determine areas within the state that have the highest prevalence.

Results: Between 2008 and 2017, 1404 blood samples from DUI and traffic crashes in Alabama were positive for methamphetamine. Methamphetamine users were predominately Caucasian (96%) and male (64%). Median (average) methamphetamine concentrations in samples collected after a DUI stop, traffic crash, and traffic death were 190 (306) ng/mL, 200 (367) ng/mL, and 295 (809) ng/mL, respectively. The highest methamphetamine blood concentrations after a DUI stop was 3,300 ng/mL. Common signs and symptoms noted during DRE evaluations included: increased physiological parameters such as heart rate, blood pressure, and temperature, dilated pupils, restlessness, and rapid movement and speech.

Conclusion/Discussion: The concentration of methamphetamine found in drivers was ≥ 50 ng/mL, ≥ 100 ng/mL, and ≥ 200 ng/mL in approximately 83%, 68%, and 48% of the cases, respectively. The vast majority of methamphetamine driving cases were not consistent with low dose administration. However, users with methamphetamine at low blood concentrations routinely displayed poor driving and impairment highlighting the ability of methamphetamine to cause impairment in both the rush or crash phase. Interestingly, we have observed an increase in "cognitive performance enhancement" legal defenses in DUI cases over recent years. The opinion that methamphetamine improves driving has been offered by hired defense experts, often irrespective of driving behavior or blood concentration. The data from this study and highlighted case studies will help address such claims. In conclusion, methamphetamine, is capable of producing severe driving impairment, and efforts to educate law enforcement, attorneys, and the public are encouraged.

Keywords: Methamphetamine, DUID, Human Performance

S4

Gabapentin in Drugged Driving Investigations

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Background/Introduction: Gabapentin (Neurontin®) has been approved by the FDA for adjunctive therapy in the treatment of epilepsy and neuralgia, but is increasingly being prescribed for multiple off-label uses including insomnia, anxiety, migraine, and as an adjunct in pain management. Side effects include somnolence, dizziness, ataxia, nystagmus, and fatigue. There are previous reports of impaired driving attributed to gabapentin (1). The number of laboratories reporting gabapentin in their 10 most frequently detected compounds/classes of drugs increased from one in 2013 to five in 2017 (2).

Objective: This presentation will review the prevalence and impact of gabapentin in forensic toxicology DUID casework submitted to NMS Labs from 2015-2017, and report gabapentin concentrations in single drug and polydrug cases.

Method: Gabapentin is not included in the routine ELISA/LC/TOF-MS drug screen offered by NMS Labs in DUID cases. A targeted LC-MS/MS test is ordered in cases where the history indicates gabapentin use or when drugs detected in the routine scope cannot account for the suspect's behavior. Between 2015 and 2017, gabapentin testing was ordered in 179 DUID cases. The LC-MS/MS method has a LOQ of 1.0 mcg/mL and this did not change over the course of this review.

Results: The number of tests ordered, positivity rate, mean (±SD), median and range of concentrations is shown in Table 1. Gabapentin was the sole drug identified in 24 cases (13%). The mean (±SD), median and range of concentrations in those cases were 10.3 ± (10.6) mcg/mL, 7.4 mcg/mL, and 1.0-44.0 mcg/mL, respectively. A review of those cases revealed "erratic driving" was the most commonly reported driving behavior. In the remaining cases, the drugs most frequently found in combination with gabapentin were opioids/opiates (38%), benzodiazepines (36%), antidepressants (27%), and marijuana (19%).

Table 1. Gabapentin positivity and concentrations

Year	Gabapentin tests ordered	Percent positive	Conc. (mean, ±SD)	Conc. median	Concentration range
2015	58	77%	9.2 ± 8.0 mcg/mL	7.1 mcg/mL	1.9-44.0 mcg/mL
2016	89	73%	9.1 ± 7.2 mcg/mL	9.0 mcg/mL	1.5-43.0 mcg/mL
2017	77	89%	9.5 ± 7.6 mcg/mL	7.4 mcg/mL	1.0-31.0 mcg/mL
TOTAL	224	79%	9.3 ± 7.5 mcg/mL	7.3 mcg/mL	1.0-44.0 mcg/mL

Conclusion/Discussion: This review found that the frequency of ordering gabapentin testing in DUID cases increased between 2015 and 2017. The positivity rate also increased during this period with an overall positivity rate of 79%. The mean and median concentrations of gabapentin were significantly higher than those reported in therapeutic use of trough plasma gabapentin concentrations of 2.6 mg/L following a dose of 900 mg/day, and an average concentration of 4.8 mg/L in patients receiving 1800 mg/day, sampled randomly. Approximately 87% of the cases in this review involved gabapentin in combination with other drugs.

Keywords: Gabapentin, drugged-driving, forensic toxicology

References

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Loperamide and desmethylloperamide concentrations in clinical and forensic blood samples during a period of increased loperamide abuse and misuse

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Background/Introduction: Loperamide, a potent μ opioid agonist, is available over the counter or by prescription for the treatment of diarrhea. The drug is a substrate for p-glycoprotein in intestinal cells where it reduces loperamide transport into the blood and across the blood-brain-barrier such that its penetration into the central nervous system is reduced, limiting psychoactive effects. Recommended doses are less than 16 mg/day. Although considered safe and without abuse potential at recommended doses, high loperamide doses are being increasingly self-administered to treat opioid withdrawal or to produce an opioid high. This effect can be increased if inhibitors of p-glycoprotein or cytochrome P-450 3A4 enzymes, (the major enzyme in loperamide metabolism) are consumed with loperamide. National Poison Control reports document an approximate doubling of loperamide cases from 2009 to 2015, with accompanying cardiorespiratory toxicities and neurological effects. The FDA released two warnings in 2016 and 2018 about potential high dose loperamide abuse and serious cardiac toxicity including QT interval prolongation, Torsades de Pointes or other ventricular arrhythmias, syncope, and cardiac arrest. In light of increasing loperamide abuse and misuse, we investigated concentrations of loperamide and desmethylloperamide, its inactive metabolite, in clinical and forensic blood samples submitted to NMS Labs, Inc. from 8/27/16 to 3/28/18.

Objective: To evaluate and report blood loperamide and desmethylloperamide concentrations in clinical and forensic samples over the last 19 months.

Method: Query the NMS Labs, Inc. LIMS database for the age and sex of individuals tested for loperamide and metabolite blood concentrations.

Results: The mean, median (range) of positive loperamide concentrations (10 ng/mL) in 85 clinical serum/plasma/blood samples were 54.5, 38.0 (11-240) ng/mL; 51.8% of samples submitted had no measurable loperamide. Males provided 57.5% and females 42.5% of the samples, and the ages of individuals were 31.2, 32.0 (0-50) years. Positive desmethylloperamide concentrations (10 ng/mL) in the clinical samples were 178, 140 (10-620) ng/mL, and 22.4% of the samples submitted had no measurable loperamide metabolite. The mean, median (range) of positive loperamide concentrations in 697 forensic blood samples were 213, 130 (10-9400) ng/mL; 46.3% of the samples submitted had no measurable loperamide. Males provided 57% and females 43% of the samples, and the ages of individuals were 46.0, 44.0 (6-94) years. Desmethylloperamide concentrations in the clinical samples were 560, 340 (10-10,000) ng/mL, and 27.3% of the samples submitted had no measurable loperamide.

Conclusion/Discussion: Median loperamide concentrations in clinical and forensic blood samples were 12 and 43 times higher than recommended therapeutic concentrations of up to 3 ng/mL, respectively. However, Kim et al. reported a peak loperamide plasma concentration of 4.3 ng/mL after a 16 mg loperamide dose (Br J Clin Pharmacol. 2014;78:556-64). The high median blood loperamide concentrations support that loperamide abuse and misuse are increasing, and these increased concentrations are accompanied by serious cardiorespiratory toxicity and in some cases death. In the investigation of suspected opioid overdoses, loperamide intoxication should be considered.

Keywords: Loperamide, Desmethylloperamide, Blood concentrations

An outbreak of brodifacoum-laced synthetic cannabinoids in the United States – Analytical and practical concerns for the toxicology laboratory

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Background/Introduction: In the first quarter of 2018, a rash of cases involving individuals seeking medical care for unexplained bleeding appeared primarily in the mid-west part of the US. Subsequently, additional cases in other geographies were reported. Based on clinical signs and laboratory-based hemostasis markers, blood from a sentinel case was sent for an anticoagulant screen. Using an LC-MS/MS-based analysis, the blood specimen tested positive for the superwarfarin brodifacoum. An excess of 100 additional submissions from patients with similar clinical signs also tested positive for brodifacoum, often accompanied by small amounts of difenacoum and bromadiolone. One patient who left care against medical advice subsequently died. The only commonality amongst the patients was the use of synthetic cannabinoid products proximate to the time of seeking medical care. Testing of some synthetic cannabinoid products by law enforcement laboratories confirmed the presence of brodifacoum. For the toxicology laboratory, it was imperative to ramp up an infrequently requested test to run every day to accommodate patient care and epidemiological concerns. Coordinating logistical issues from all the submitting agencies was challenging given the widespread geographical locations where patient care occurred. Nevertheless, the coordinated efforts of governmental agencies, both federal and state, and the testing laboratory were key in identifying the cause of bleeding in affected individuals as well as the scope of the problem.

Objective: To highlight the role of the toxicology laboratory in addressing a public health and safety crisis of toxicological origin. Additionally, to review the toxicology of superwarfarin anticoagulants and their analysis in biological matrices.

Method: Blood and serum/plasma samples from affected patients were received from hospitals, departments of health and medical examiner offices and analyzed for anticoagulants. Additionally, a few patients had paired analyses for synthetic cannabinoids. The assay can identify brodifacoum, bromadiolone, chlorophacinone, dicumarol, diphacinone and warfarin. As deuterated internal standards for the majority of compounds were not available, the assay was performed qualitatively and employed standard addition. Following addition of chloro-warfarin as an analog internal standard, an acetonitrile crash was followed by a back-extraction into MTBE. After dry down, an aqueous ammonium hydroxide/methanolic ammonium hydroxide mixture was used to reconstitute the extract. LC-MS/MS analysis employed negative-ion electrospray and utilized a C18 column with aqueous ammonium hydroxide/methanolic ammonium hydroxide mobile phase. A 10 ng/mL standard was used to verify the reporting limit. While results were reported qualitatively, it was possible to get relative concentrations from patient to patient. Analysis for synthetic cannabinoids was previously reported (Tynon M et al. 2017. Drug Testing Anal; 9 (6): 924-934).

Results: Standard addition analyses identified in excess of 100 cases positive for brodifacoum in the affected cohort of patients. In addition to brodifacoum, many patients also had relatively smaller amounts of difenacoum and bromadiolone. The presence of these superwarfarins was consistent with the clinical signs and laboratory-based hemostasis testing of the patients. Brodifacoum is a potent anticoagulant belonging to the warfarin family of compounds. It exerts its effects via inhibition of the Vitamin K cycle, thus resulting in depressed synthesis of clotting factors. A few cases of paired analyses for synthetic cannabinoids confirmed the presence of these substances in blood or serum/plasma. The primary synthetic cannabinoids detected in the limited paired cases were AB FUBINACA and 5F-AMB.

Conclusion/Discussion: Public health and safety crises involving toxicological agents occur from time to time. The toxicology laboratory is critical in identifying causative agents. In such a time, the laboratory must adapt to the exigent needs by accommodating more frequent testing and providing continuous data to assist in epidemiologic evaluations and, ultimately, assessing the depth and gravity of the situation. In this case, brodifacoum was identified in a cohort of patients with the only commonality being use of synthetic cannabinoid products proximate to clinical signs and symptoms. These findings were critical to supporting treatment of affected patients and, in at least one case, confirming the cause of death.

Keywords: brodifacoum, synthetic cannabinoids, analysis

Analysis of Pesticides in African Wildlife Poisonings

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Background/Introduction: The use of pesticides as poisons for the illegal killing of wild animals in Africa has become more widespread due to the ease of access to pesticides and their acute toxicity. The motive for these poisonings is frequently to prevent wildlife from foraging on crops or eating domesticated animals. Despite an increase in awareness, education and global attention to this issue, killing wildlife with the use of poisons has become more problematic, as the toxins used can be transferred up the food chain, and potentially impact higher species including humans. These ingestions create additional risks for species already on the brink of extinction.

Objective: The objective of this research was to develop a qualitative method for the collection of samples in rudimentary field conditions, and to toxicologically confirm the presence of organophosphates, carbamates, and selected other pesticides in African wildlife samples from Zimbabwe and other nations in an effort to provide forensic evidence to wildlife advocates and local authorities.

Method: A method was developed using a Waters Acquity I Series Ultra Performance Liquid Chromatograph® coupled to a Waters Xevo® TQD mass spectrometer to detect aldicarb, aldicarb sulfone, aldicarb sulfoxide, carbaryl, carbofuran, 3-hydroxycarbofuran, methomyl, monocrotophos, and strychnine in bait and animal remains. The method was validated qualitatively to determine limit of detection, carry-over, recovery, and stability.

Samples were collected in the field by members of the Wildlife Unit in the Department of Veterinary Services in Zimbabwe and consisted of bait, stomach, intestinal and crop contents, muscle, urine, liver, serum, and tissue. The samples were extracted in the field using a basic liquid extraction developed at the CFSRE employing readily available supplies that could be purchased at a local hardware store. The samples were soaked in acetone for at least 10 minutes and filtered through a funnel containing a paper towel. The filtrate was collected into a glass jar, then poured onto a clean paper towel and air dried. An extraction blank was collected in the same manner without any biological matrix. The dried paper towels were shipped via DHL Express to the United States for analysis, arriving within 7 days. A cutting of the paper towel was taken, immersed in ethyl acetate (5mL), vortex mixed, and allowed to passively equilibrate for 10 minutes. The eluent was decanted, evaporated to dryness, and reconstituted in 0.5 mL of mobile phase. The samples were then centrifuged to remove any paper towel particulate. Chromatographic separation was achieved using an Acquity UPLC® BEH C18 column (2.1 mm x 100 mm x 1.7 um) and ammonium formate (pH 4) and 0.1% formic acid in methanol mobile phases for a total run time of 5 minutes.

Results: Samples were collected from 16 animals: knob-billed goose (n=1), warthog (n=1), elephant (n=9), vulture (n=2), hippopotamus (n=2), and buffalo (n=1). Additionally, three baited fruit samples, maralua fruit (n=2), and watermelon (n=1), were collected. Sixteen extracted blank paper towels were received as controls. Several samples were demonstrated to contain carbofuran, aldicarb, and monocrotophos. Samples that contained one or more of these compounds included marula fruit, watermelon, hippo stomach, buffalo liver and stomach, elephant stomach contents, vulture stomach contents and liver, knob-billed goose crop contents and liver, and warthog liver and serum.

Conclusion/Discussion: The in-field extraction shows promising results as a way to export these specimens for analysis that would not be able to be performed in Africa due to the lack of laboratory facilities in the field. The analytical method has proven to be sensitive enough to identify the target analytes within a wide range of matrices using this simple crude extraction. Using results from these analyses, connections can be made to aid in the investigation and apprehension of individuals involved in the illegal killing of these endangered species. In addition, these results provide clear evidence that can be used to help make these toxic compounds deregistered in countries where they are abused.

Keywords: African Wildlife, Pesticides, LC/MS/MS

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In Vitro Inhibition of Opioid Metabolism by Benzodiazepines

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Background/Introduction: We have been conducting a series of studies to determine compounds that may inhibit the metabolism of the opioids buprenorphine, methadone and oxycodone. While the opioids are dangerous in their own right, we hypothesize that inhibition of metabolism could increase their plasma concentration to toxic levels, and in the case of oxycodone metabolism to oxymorphone it may also decrease the therapeutic effect.

Objective: The goal of this work was to establish in vitro IC50 values (or IC50 estimates from extrapolations when solubility was limiting) for benzodiazepine class drugs.

Method: Initial human liver microsome (HLM) screens were conducted to identify potential drug interactions and establish inhibitor concentration estimates for IC50 determination. Potential inhibitors at 3 different concentrations were incubated with HLM. The opioids were added along with or 15 minutes after the inhibitor and a source of NADPH (- and + pre-incubation, respectively) to test for time-dependent inhibition (TDI). The results of the screens were then used to identify which opioid/benzodiazepine combinations would be incubated with human CYPs to determine IC50 values. For presumptive IC50 concentrations below the solubility limit, a 10-concentration inhibition assay was performed with the respective CYP and opioid. If the presumptive IC50 was close to the solubility limit, a three-concentration assay was designed to permit potential extrapolation of an IC50. For several pairings, an IC50 > solubility limit was set.

Results: Screens in HLM suggested the following experiments would be performed:

Benzodiazepine	Buprenorphine	Methadone	Oxycodone to Noroxycodone	Oxycodone to Oxymorphone
Alprazolam	extrapolation	>400	>400	>400
Clonazepam	extrapolation	IC50	extrapolation	IC50
Demoxepam	>20	>20	>20	>20
Diazepam	extrapolation	extrapolation	extrapolation	>150
Estazolam	IC50	>80	IC50	>80
Flunitrazepam	extrapolation	extrapolation	extrapolation	extrapolation
Flurazepam	IC50	IC50	IC50	IC50
Lorazepam	>200	extrapolation	IC50	IC50
Midazolam	IC50	IC50	IC50	IC50
Nitrazepam	extrapolation	extrapolation	>125	>125
Norchlordiazepam	>25	>25	extrapolation	>25
Nordiazepam	extrapolation	>20	IC50	IC50
Oxazepam	extrapolation	extrapolation	>10	extrapolation
Temazepam	extrapolation	>200	extrapolation	extrapolation
Triazolam	IC50	>80	>80	>80

IC50 results for select CYP450s/benzodiazepines:

CYP/Substate/Inhibitor	$[I]_{pl}$ (μM)	IC50 (μM)		K_m	K_i		AUC_i/AUC_n	
		-	+		-	+	-	+
3A4/Buprenorphine/Midazolam	0.31	21.2	0.922	13.6	8.58	0.37	1.04	1.84
3A4/Buprenorphine/Flurazepam	2.58	44.8	29.8	13.6	18.13	12.06	1.14	1.21
3A4/Oxycodone/Midazolam	0.31	1.11	0.617	600	1.07	0.6	1.29	1.52
3A4/Oxycodone/Flurazepam	2.58	1.30	3.58	600	1.26	3.46	3.05	1.75

Conclusion/Discussion: IC50 results indicate that certain benzodiazepines are capable of significant inhibition of some CYP-mediated pathways. Extrapolating the in vitro values to in vivo values using the Cheng-Prusoff equation, $K_i = IC_{50} / (1+S/K_m)$, and an in vivo extrapolation equation, $AUC_i / AUC_n = 1 + [I]_{pl}/K_i$, demonstrates that several incubation combinations led to drug interactions of potential clinical interest. Ratios of $AUC_i / AUC_n \geq 2$ indicate potentially significant inhibitions. The inhibition of CYP3A4/oxycodone metabolism by flurazepam exceeded this guideline. The inhibition of CYP3A4/buprenorphine metabolism by midazolam was below the guideline, but with pre-incubation the AUC_i / AUC_n ratio was high enough to warrant concern. The significant lowering of the IC_{50} with pre-incubation indicates that a metabolite of midazolam was responsible for the inhibition, at least in part.

Funding: This research was supported by a grant from the National Institute of Justice, 2015-IJ-CX-K009.

Keywords: Opioids, Drug Interactions, Benzodiazepines

Use of Gabapentin in a Series of Sexual Assaults

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Background/Introduction: Gabapentin has been in use since 1986 as an anticonvulsant and for treatment of neuropathic pain. Until recently, it has not been considered a particularly dangerous drug or a potential drug of abuse. Many clinical and forensic toxicology programs do not routinely test for it. Recently, however, reports have circulated that illicit use of gabapentin has been gaining in popularity, most likely for its analgesic and sedative properties. In 2017, Kentucky became the first state to list gabapentin as a controlled substance. Ohio's Substance Abuse Monitoring Network has issued a warning of misuse for gabapentin, and the federal Food and Drug Administration is currently considering reconsidering its abuse potential. Presented here is a case of gabapentin being used in a series of sexual assaults by a perpetrator familiar with not only its chemical properties, but with the potential to evade discovery during laboratory testing.

Objective: To examine the potential and incidence of abuse of gabapentin, and the possibility of its use in sexual assault cases.

Method: The case record examination includes court transcripts, police reports, and laboratory reports from the Michigan State Police and Mercy Hospital Muskegon. Literature reviews of the abuse potential of gabapentin are also presented.

Results: This is a case of the classic scenario of an acquaintance putting something in the drink of an unsuspecting person. Over 20 victims reported similar events with the same two suspects. Victims came in contact with the two defendants on dating websites. They met one of the two at a bar or restaurant; the other then showed up. Victims reported feeling dizzy, nauseous, sedated after consuming drinks purchased by the defendants. They were taken by the defendants to one of their houses, where they were sexually assaulted. The defendants claimed all sexual encounters were consensual. Several victims had hospital drug tests performed but the results were negative for typical immunoassay panels and GHB. Urine alcohol results ranged from zero to > 0.10 g/dL but no confirmatory testing for drugs other than GHB was done. One case was sent to the Michigan State Police where solid phase extraction followed by full-scan GC/MS analysis detected the presence of gabapentin and amitriptyline in the urine of the victim. Urine alcohol was measured by headspace gas chromatography as 0.02 g/dL. The victim had no prescriptions for the drugs and denied usage. Hospital tests performed on the urine of other victims did not include testing for tricyclic antidepressants or gabapentin. One of the suspects worked in a pharmacy where it was speculated he had obtained the drugs. Recent literature reviews show that gabapentin has more abuse potential than was previously thought and suggest it should be monitored more closely.

Conclusion/Discussion: Gabapentin may have more abuse potential than previously realized, and is a potential agent for drug-facilitated sexual assault. Routine emergency room drug tests do not target gabapentin, and a knowledgeable predator may take advantage of this fact to avoid detection. Federal and state governments should consider whether use of gabapentin should be more tightly controlled. Hospitals and crime laboratories investigating drug-facilitated sexual assaults should routinely test for the presence of gabapentin as a potential incapacitating agent.

Keywords: Gabapentin, amitriptyline, drug-facilitated sexual assault

Blood and urine zopiclone kinetics after a single dose

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Background/Introduction: The sleeping aid zopiclone is a frequently prescribed drug and it is also commonly found in forensic contexts such as drugs and driving cases as well as some cases of drug facilitated sexual assault. However, data on the blood and urinary pharmacokinetics of zopiclone and its metabolites are scarce. In an effort to fill this knowledge gap and to investigate the metabolite excretion profiles we designed a controlled study using two doses of zopiclone in healthy volunteers.

Objective: To describe the blood and urine parent drug and metabolite pharmacokinetics of a single dose of zopiclone.

Method: 16 healthy volunteers were administered either 5 mg (N=8) or 10 mg (N=8) of zopiclone. The protocol was approved by the regional ethics committee in Linköping. All subjects provided written informed consent to participate. After the drug administration, blood samples were obtained 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 24, and 48 hours post dose. Urine samples were obtained after 2, 4, 6, 8, 10, 12, 14, and 24 h and then after 2, 3, 4, 5, 6, 10, and 14 days post dose. Two analytical methods previously described and published were used to determine zopiclone and desmethylzopiclone in blood and zopiclone, desmethylzopiclone, and zopiclone N-oxide in urine. The technique used was tandem mass spectrometry and the lower limits of quantitation (LLOQ) were 1 ng/g blood and 5 ng/mL urine.

Results: There was a significant difference between the peak concentration of both zopiclone and desmethylzopiclone in blood from the 5 mg and the 10 mg doses indicating a linear dose concentration relationship. Mean peak zopiclone concentrations were 29 and 58 ng/g for 5 and 10 mg and for desmethylzopiclone they were 4.4 and 8.6 ng/g, respectively. With an LLOQ of 1 ng/g no sample was positive at 48 hours, and with the exception of one subject, all were positive for zopiclone or its metabolite at 24 hours post dose. During the first 10 hours after dosing, the zopiclone concentration exceeded that of desmethylzopiclone.

Among the measured urinary analytes, the N-oxide was the primary metabolite which dominated during the first 6 hours post dose with peak creatinine corrected concentrations between 900 and 5260 ng/mg and no significant differences between doses.

The parent zopiclone showed concentrations between 230 and 3290 ng/mg creatinine and significant concentrations of zopiclone was found up to 24 hours post dose in all subjects, up to 48 h in 4 subjects, and 72 hours in 1 subject. The longest detection times were found for the desmethyl metabolite with 2 subjects presenting with positive samples after 120 and 144 hours. The concentration range for desmethylzopiclone was 480 to 2710 ng/mg creatinine. The kinetics of zopiclone N-oxide and desmethylzopiclone differed so that after 12 hours the concentrations of desmethylzopiclone exceeded those of the N-oxide in all subjects. Thus, the relationship between the two metabolites may be used to estimate the time of intake.

Conclusion: We conclude that after a single dose, zopiclone was detected in blood up to 24 hours and that desmethylzopiclone could be detected up to 6 days in urine and seems to be a good analyte to investigate zopiclone intake even if several days have passed since intake. In addition, the data suggest that the ratio between zopiclone N-oxide and desmethylzopiclone can be used to narrow the estimated time of intake.

Keywords: zopiclone, pharmacokinetics, detection times, blood, urine

The Hidden Costs of the Opioid Crisis and the Implications for Financial Management for Forensic Laboratories — How Does a Laboratory Document its Economic Impact?

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Background/Introduction: West Virginia University's Project FORESIGHT is a self-evaluation of U.S. crime laboratories. Laboratories voluntarily participate by providing laboratory casework, personnel, and budget costs data which is used to calculate their average cost per case, cost per test and other metrics that help leverage resources and understanding of laboratory efficiency. The Bureau of Justice Statistics' Census of Publicly Funded Forensic Crime Laboratories provides a complimentary resource in understanding the costs associated with completing casework in the forensic laboratories. With the increase in opioid related crimes and deaths, a better understanding of forensic laboratory cost analysis is needed.

This research provides laboratories with a means to document the increased financial burden associated with the increase in opioid related crimes and deaths. Prior to November 2017, the magnitude of the national opioid crisis was estimated to cost nearly 0.33% of Gross Domestic Product (GDP). However, the release of the White House report (The Council of Economic Advisers, 2017) on the opioid crisis suggests that indirect costs, not previously considered, increase annual cost estimates by 597% to an annual cost of $504 billion, or 2.2% of annual GDP (Florence, Zhou, Luo, & Xu, 2016). When those considerations are examined at the individual state level, the "crisis" states (i.e., the states with the highest per capita overdose deaths) experience a cost approaching 15% of Gross State Product. This report offers a "20,000-foot view" of the societal costs from opioid abuse. The costs include medical care, substance abuse treatment, workplace costs, criminal justice costs, and opportunity costs from preventable deaths.

Objective: Project FORESIGHT data combined with the 2014 Census of Publicly Funded Forensic Crime Laboratories data were evaluated to identify the magnitude of the ongoing U.S. drug problem and identify trends with productivity, turnaround time, backlogs, queuing elasticities of demand (supply and demand models), and related econometric calculations.

Method: Following discussion of the White House report, this presentation will provide an overview of data available to estimate costs of forensic laboratories in the justice system. Additionally, it will provide a brief overview of relevant work in criminal justice support systems, cost structure, economies of scale, policy implications, elasticities, and other items relevant to updating apportionment estimations. Some trends can be investigated at a jurisdictional level to better address local and national policy. A view of nationwide trends compared to jurisdictional trends, specifically in jurisdictions most severely impacted by the opioid crisis will be discussed. Since the White House report details a single year, the analysis takes a static view of this dynamic, growing problem. The resources required to address the opioid crisis must be aimed at a moving target, rather than focused and addressed retrospectively.

Results: The dynamics show a growing national trend in "opioid" states, representing ten states with the highest per opioid overdose deaths. Further, efforts to reduce turnaround time in the laboratory show that requests for analysis outpace capacity. This presentation will discuss the dynamic trends in opioid costs such as time series, a "National" vs "Opioid states" picture, complexities of "hitting" the moving target, and queuing the elasticities of demand.

Conclusion/Discussion: The estimated cost for the criminal justice system to deal with the opioid crisis is approximately $78 billion. However, the foundations for the criminal justice system costs are rough approximations of system-wide costs; they offer little advice at the jurisdictional level to manage scarce resources dealing with the crisis. Additionally, the White House Report cost estimates do not consider the indirect costs borne by individual forensic laboratories. This research provides a more accurate societal costs impact that can better reflect the economic impact beyond previously calculated estimates.

Keywords: Opioid Crisis, Economic Impact, Forensic Laboratory Cost Analysis

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Monitoring Changes in Novel Psychoactive Substances Using a Population of Electronic Dance Music Festival Attendees from 2014-2017

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Background/Introduction: Novel psychoactive substances (NPS), ingested for their euphoric and stimulating effects, have become widely circulated at electronic dance music (EDM) festivals and have had several adverse events associated with their use at festivals in the United States (US). Many of the products have been found to contain a variety of unregulated synthetic stimulants that includes phenethylamines and cathinones. The range of NPS drugs currently available on the market has continued to grow, which has resulted in analytical challenges within the forensic community in detecting and monitoring novel drug use.

Objective: The objective of this research was to conduct a longitudinal study of recreational drug use by attendees at three major EDM festivals (Miami, Tampa and Atlanta) in the US between 2014 and 2017. Goals of the project included evaluating the utility of monitoring an at-risk population for detection of NPS and evaluating trends within this population to provide information about the emergence and turnover of NPS to the forensic community.

Method: Participants were peer-recruited at each festival. After obtaining informed consent, participants were asked a series of open-ending questions related to their drug use within the last week. An oral fluid sample was then collected using the Immunalysis Quantisal®. Samples were initially screened using high resolution mass spectrometry and subsequently confirmed using liquid chromatography tandem mass spectrometry.

Results: Between six sample collection events over four years, 1,233 oral fluid samples were obtained. Self-reported survey responses suggest as many as 66% of attendees at the events admitted to recent drug or alcohol use. Of the 1,233 oral fluid specimens collected in the study, 352 (28.5%) confirmed positive for a novel stimulant, MDMA, and/or MDA. With respect to any positive oral fluid samples, including drugs of abuse and/or NPS (n=684), the positivity rate for a novel stimulant, MDMA, and/or MDA was 51.5%.

Comparing four years of data collected from a single festival site (Miami), temporal trends demonstrated high rates of turnover with respect to novel stimulants, and a resurgence in MDMA positivity in recent years. Novel stimulants detected with the highest positivity rates in 2014, like alpha-PVP, methylone and dimethylone, either were never detected again (alpha-PVP) or significantly decreased in positivity in subsequent years. Dibutylone positivity peaked in 2016 and N-ethyl pentylone positivity increased in 2017. Positivity rates also generally increased with each day of the festival, with the final day having the highest positivity for NPS. The data did not demonstrate any differences between gender or within age groups for novel stimulant and/or MDMA positivity.

In comparing the trends of novel stimulant and MDMA positivity across three geographic locations over four years, it was hypothesized there would be differences, which might be attributed to drug trafficking patterns and/or regional variability. Small differences in positivity rates were detected but could not be attributed solely to the geographical location. N-ethyl pentylone positivity decreased in Atlanta (September 2017) compared to Tampa (May 2017), which could either reflect regional differences or a shift back to MDMA. MDMA and MDA positivity rates were both relatively stable across all three locations.

Conclusion/Discussion: Across all years, the diversity of novel stimulants confirmed in biological specimens continued to evolve and a new stimulant was detected with the highest positivity rate each year of the study. Based on the data from this study, the life cycle of novel stimulant drugs run a course of a year or less, and new drugs appear and disappear over the course of two years or less. These results stress the importance of updating laboratory-based methods for the detection of emerging drugs, the ability to be able to distinguish between isomeric NPS, and the utility of this target population for monitoring trends.

Keywords: NPS, oral fluid, trends

PLATFORM

What's in the Box? – Identifying Electronic Dance Music Festival Amnesty Box Drugs

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Background/Introduction: Electronic dance music (EDM) first gained popularity in underground rave parties typically held in secret locations. In the early 1990s, these rave parties began to legitimize into organized concerts which allowed EDM to become more mainstream. EDM concerts have now become multi-day festivals with some drawing well more than 100,000 attendees. Recreational drug use has long been associated with EDM festivals with methylenedioxymethamphetamine (MDMA) being the drug of choice. In 2016, an EDM festival in Hillsborough County had two fatal overdoses related to MDMA use. Subsequently, concert promoters and law enforcement became more focused on safety and reducing drug use. Amnesty boxes, which allow concertgoers to relinquish drugs found on them with no legal repercussions, are currently being used to deter drug use at EDM festivals.

Objective: To categorize, photograph and identify drugs and drug paraphernalia surrendered to the amnesty box of a local EDM festival. To identify drug trends at EDM festivals.

Method: The confiscated drug materials were classified into groups: powders, capsules, tablets, edibles, liquids, pipes, blotter papers and mushroom plant material. Edibles and tablets were further sorted into groups based on likeness and capsules were sorted by size and visual examination of the contained material. One sample from each group was chosen for analysis unless there was an indication that the group may not be homogenous. One piece of blotter paper was chosen for analysis when an item contained multiple blotters. All powders, liquids/paraphernalia and mushroom plant material were analyzed. The powders, capsules, tablets and blotter papers were dissolved in methanol. The mushrooms were soaked in methanol overnight. Edibles and liquids were analyzed using a liquid-liquid basic drug extraction and pipes were rinsed with hexane. All samples were analyzed by full scan gas chromatography mass spectrometry (GCMS).

PLATFORM

Results:

322 tablets and capsules with MDMA	7 bags methamphetamine powder
123 hits of blotter paper with LSD	3 bags N-ethyl pentylone powder
90 tablets and capsules of over the counter and prescription medications	3 capsules with 4-MEAP and N-ethyl pentylone
60 tablets and capsules with MDA	3 chocolates with Psilocin
42 capsules with N-ethyl pentylone	3 tablets with MDMA and sildenafil
22 psilocin mushrooms	1 capsule with 4-MEAP
22 capsules with mitragynine	1 tablet with BZP, TFMPP, ethylone, and MDPV
20 tablets with amphetamine	1 capsule with ethylone
19 bags MDMA powder	1 bag ethylone powder
16 bags cocaine powder	1 bag of gummies containing alprazolam
12 alprazolam tablets	1 chocolate with THC
11 pipes and vapor cartridges with THC	1 rainbow gummy with LSD, cocaine and N-ethyl pentylone

Conclusion/Discussion: The most commonly identified drugs were MDMA and LSD. Analysis of the amnesty box drug materials revealed that synthetic cathinones, methamphetamine and MDMA look very similar visually and tablets did not always contain purely MDMA. There was a high prevalence of N-ethyl pentylone and users may not realize what drugs they are actually taking which can lead to overdose situations. Analyzing amnesty box contents can give toxicologists an idea of what drugs are prevalent locally providing guidance for future laboratory procedures and casework.

Keywords: Electronic Dance Music, Amnesty Box, MDMA

Phase I Metabolism of Mitragynine using In Vitro Methods

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Background/Introduction: *Mitragyna speciosa* (Kratom) is a psychoactive plant that has recently emerged as a recreational drug in the United States. Although the plant contains numerous alkaloids, mitragynine is the principal pharmacologically active compound. 7-Hydroxymitragynine, which is a more potent μ-receptor agonist, is also present in the leaves of the plant at much lower concentrations. Oxidation and hydroxylation of mitragynine are reported to be the primary metabolic transformations during phase I metabolism. Mitragynine has been reported to inhibit CYP3A4, CYP2D6, and CYP2C9. In addition, mitragynine has also been reported to induce CYP1A2 and CYP3A4 at the transcriptional level. Studies also suggest that mitragynine could have potential drug-drug interactions with substances that are P-glycoprotein substrates. Despite growing interest in the drug, no studies to date have explored the role of CYP isoforms responsible for metabolism. This information can help predict the impact of genetic polymorphisms and the potential risk of drug-drug interactions**.**

Objective: The goal of this study was to investigate the in-vitro metabolism of mitragynine using eight recombinant cytochrome P450 isoenzymes (rCYP1A2, rCYP2B6, rCYP2C8, rCYP2C9, rCYP2C18, rCYP2C19, rCYP2D6 and rCYP3A4) expressed in *E. coli* (bactosomes).

Method: Liquid chromatography-quadrupole/time of flight-mass spectrometry (LC-Q/TOF-MS) was used to investigate the in-vitro metabolism of mitragynine. Recombinant human cytochrome P450 enzymes were incubated separately to evaluate metabolic activity towards mitragynine (t = 0, 60, 120, 180 and 240 minutes). Control and blank samples were included in each assay. Potential metabolites were qualitatively identified using accurate mass measurements (chemical formula) and MS/MS spectra (structural information). Isoenzymes with observed activity were further investigated using known inhibitors. rCYP2C18, rCYP2C19, rCYP2D6, and rCYP3A4 were incubated side by side, in the presence and absence of inhibitor (n=4 or 6). Ketoconazole (20 μM) was used for rCYP2C18, rCYP2C19 and rCYP3A4 and fluvoxamine (20 μM) was used for rCYP2D6.

Results: An in-vitro study using rCYP enzymes suggests that the biotransformation of mitragynine involves 2C18, 2C19, 2D6 and 3A4 isoenzymes. Three known phase I metabolites were identified, including 16-carboxymitragynine (rCYP2C18, rCYP2C19, and rCYP2D6), 9-O-demethylmitragynine (rCYP2C19, rCYP2D6 and rCYP3A4), and 9-O-demethyl, 16-carboxymitragynine (rCYP2C19). In addition, the psychoactive compound 7-hydroxymitragynine was also identified as a metabolite (rCYP3A4). Inhibitors and replicate analyses were used to confirm these findings.

Conclusion/Discussion: As many as four CYP isoenzymes may be involved in mitragynine metabolism. In addition to previously reported carboxylated and demethylated species, 7-hydroxymitragynine was also identified as a novel metabolite. Inhibition studies using ketoconazole confirmed that CYP3A4 was responsible for this biotransformation. Isoenzymes involved in drug metabolism can provide important insight in terms of toxicity and the potential for drug-drug interactions. Given that polydrug use is common among kratom users, additional study is warranted.

Keywords: Mitragynine, Kratom, Metabolism

XLR-11 form abundant reactive metabolites that may contribute to the observed kidney toxicity.

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Background/Introduction: XLR-11 is a synthetic cannabinoid that was first seen in 2011. It was frequently used in the United States but has also been encountered in Europe. Toxicities included vomiting, nausea and acute kidney injury (AKI) that are inconsistent with adverse effects expected from cannabis analogues. XLR-11 has been associated with fatalities and shown in a cell model to produce DNA damage. Although it has been suggested that the observed AKI is linked to the cannabinoid receptors, a clear and exhaustive description of the underlying mechanism is still missing. In this study we propose an additional mechanism that may contribute to the toxicity.

The reported toxicities could also be caused by reactive metabolite (RM) formation. RMs are unstable intermediates formed during the metabolism of a drug. Often they are quickly metabolized further into more stable compounds; but sometimes they instead bind covalently to enzymes and/or DNA, disrupting cell function and leading to toxicity. When this occurs with prescription drugs, the toxicity is sometimes severe enough to cause withdrawal from the market. Screening procedures are utilized during drug development to avoid drugs that can form RMs and cause toxicity. However, no such control mechanisms are known to be employed during development of illicit drugs.

The structure and metabolism of XLR-11 indicate that RMs may be formed. Especially, highly reactive aldehyde metabolites could be formed through oxidation of the methyl groups of the TMCP ring. Although these aldehyde metabolites are difficult to detect due to their reactivity, metabolism studies of XLR-11 have revealed both monohydroxylated metabolites, which could be precursors, and carboxylic acid metabolites, which are formed via aldehydes.

Objective: The structure, metabolism and toxicity of XLR-11 all indicate the possibility of RMs and the objective of the present study was to investigate whether XLR-11 forms reactive metabolites when incubated with human liver microsomes.

Method: Potential reactive metabolites of XLR-11 were "trapped" using trapping reagents that bind to different reactive metabolites and form stable adducts. As the nature of reactive metabolites differs, glutathione (GSH), potassium cyanide (KCN) and methoxylamine (MXA) were all used. MXA is the reagent that primarily traps aldehyde metabolites.

XLR-11 (10 μM) was incubated with human liver microsomes (1 mg/ml) for 60 min at 37 °C without trapping agents, as well as with GSH (2 mM), KCN (1 mM), or MXA (2 mM) in four separate experiments. Clozapine, rimonabant and prazosine were used as positive controls for RM formation and trapping. All samples were analyzed by LC-QTOF using data-dependent acquisition. Stable metabolites as well as trapped adducts were identified by accurate mass, retention time and when available, MSMS spectra.

Results: XLR-11 formed 27 stable metabolites and 15 MXA-adducts but no GSH or KCN adducts were found. The combined peak area of the MXA-adducts corresponded to 57% of the total metabolite area after MXA incubation. The three major adducts corresponding to 84% were all consistent with a trapped aldehyde on the TMCP ring, in one case in combination with oxidative defluorination of the pentyl side chain.

Conclusion/Discussion: Although it is always precarious to estimate relative concentrations of metabolites based on signal intensity due to variations in ionization intensity, the abundance of MXA adducts is striking and would most certainly trigger a more in-depth investigation and/or redesign in pharmaceutical development. It is apparent from our data that XLR-11 forms abundant reactive aldehyde metabolites.

These RMs could interact with enzymes or DNA, and contribute to the unusual toxicity reported for XLR-11. To verify this possibility, further studies are needed to show evidence of DNA damage or covalent binding to proteins.

Keywords:Toxicity; Synthetic cannabinoids; Metabolism

Metabolite characterization of flurazepam and norflurazepam in cryopreserved human hepatocytes and authentic urine by high-resolution mass spectrometry

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Background/Introduction: Among the emergence of new psychoactive substances, benzodiazepines have recently started to see the rise on the illicit drug market, including flurazepam and norflurazepam. Flurazepam is a hypnotic marketed under the brand name of Dalmane. The metabolism was investigated a few decades ago in human and animal urine and the major human biotransformation was reported to be oxidative deamination followed by reduction (2-hydroxyethylflurazepam). Norflurazepam, which has been sold as an NPS, is the desalkyl metabolite of flurazepam. In blood, the reported half-lives of flurazepam and 2-hydroxyethylflurazepam are significantly shorter than that of norflurazepam. Distinguishing an intake of flurazepam and norflurazepam poses an important analytical challenge as the drugs might have different legal status.

Objective: Our objective was to describe the metabolism of flurazepam and norflurazepam in human hepatocytes.

Method: Norflurazepam and flurazepam were incubated with human hepatocytes at the concentration of 10 µmol/L on a 96-well plate at 37 °C for 3, 5, and 7 h. The reaction was quenched with ice-cold acetonitrile and the supernatant was analyzed by liquid chromatography–quadrupole time-of-flight mass spectrometry (LC–QTOF). In addition, 16 authentic human urine samples, whose corresponding blood samples tested positive for norflurazepam were analyzed by LC–QTOF both non-hydrolyzed and hydrolyzed with beta-glucuronidase/arylsulfatase to investigate the presence of flurazepam and norflurazepam metabolites. Chromatographic separation was performed on an Agilent 1290 Infinity UHPLC system with an Acquity HSS T3 column at 60 °C in gradient mode over 17.5 min using the following mobile phases: (A) 10 mM ammonium formate in 0.05% formic acid and (B) 0.05% formic acid in acetonitrile. Mass spectrometric data was recorded with an Agilent 6550 QTOF mass spectrometer with an electrospray ionization source in auto MS/MS acquisition mode.

Results: In the human hepatocyte experiments no metabolites were found for norflurazepam. For flurazepam, several metabolites were detected including *N*-deethylation, *N*-dideethylation, *N*-dealkylation resulting in norflurazepam, and oxidative deamination followed by reduction to 2-hydroxyethylnorflurazepam with or without glucuronidation. The most intense peak was found to be the desethyl metabolite while norflurazepam was a minor metabolite.

In the authentic urine samples, six compounds including one glucuronide was found. 3-hydroxynorflurazepam was detected in all but one sample. Norflurazepam was detected in 10 samples. In addition, three metabolites with a hydroxylation at an aromatic ring were detected. The 3-hydroxynorflurazepam was the most abundant metabolite in urine. No unique flurazepam metabolites were found.

Conclusion/Discussion: Several metabolites of flurazepam were detected in hepatocytes with most modifications on the side chain rather than the ring structures, which adds to the hypothesis that the ring structures are not readily metabolized by hepatocytes as seen for norflurazepam. Compared to previous literature where 2-hydroxyethylflurazepam was the main metabolite we found desethylflurazepam to be the most prominent. Our studies confirmed that norflurazepam is a minor metabolite of flurazepam. In the light of the existing literature the urine sample results point towards an intake of norflurazepam. However, because of the differences in half-life, the presence of norflurazepam and/or its metabolites in the absence of flurazepam unique metabolites cannot exclude the possibility of flurazepam intake.

We recommend that confirmatory methods include several metabolites to enable the correct interpretation. We suggest desethylflurazepam and 2-hydroxyethylflurazepam as proof for the intake of flurazepam. As norflurazepam is a metabolite of flurazepam, the presence of norflurazepam needs to be interpreted with caution.

Keywords: Metabolism, flurazepam, norflurazepam

Evaluation of Mitragynine Concentrations in 410 Forensic Investigations

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Background/Introduction: Despite the reported overdose deaths related to the use of an herbal supplement, "Kratom" and consequent media attention, it remains unregulated and sold online and in smoke shops. *Mitragyna Speciosa*, commonly referred to as "Kratom", is a tropical plant with a long history of use as an herbal medicine for the treatment of depression and diarrhea, among other purposes in Southeast Asia. Traditionally, the leaves are chewed or added to hot water to brew a tea. Kratom contains many alkaloids, of which mitragynine is the predominant psychoactive ingredient, although 7-hydroxymitragynine is the most potent. Mitragynine has dose-dependent stimulant and opioid effects and has gained popularity in Western society as a treatment for opioid withdrawal symptoms and an herbal adjunct to opioids for pain management, leading to significant debate regarding its use and safety.

Objective: In this study, we review blood concentrations and concomitant findings for 410 mitragynine positive cases reported by NMS Labs from September 2016 to March 2018.

Method: Mitragynine was originally reported qualitatively from toxicology analyses from an LC-MS/MS confirmation after a LC-TOF/MS screen. Due to subsequent popularity and interest in interpretation of mitragynine in forensic investigations, a quantitative method was developed with an LOD of 0.16ng/mL, and analytical range of 5.0-500ng/mL. A directed analysis was introduced in September 2016. In January 2018, the mitragynine quantitation became a part of a scope of analysis for blood expanded DUID and postmortem panels.

Results: Of 410 cases examined, mitragynine was detected by a postmortem screen panel via LC-TOF/MS and confirmed by LC-MS/MS in 41% (n=168); 58% (n=236) were submitted as a direct mitragynine quantitation without general screening. A small subset (n=6; 1.5%) was identified as DUID cases. Mitragynine blood concentrations greater than 100ng/mL were reported in 57% of cases. The most frequently reported concentrations were >100–500ng/mL, which accounted for the 1/3 of the total quantitative results followed by >500–1,000ng/mL. When gender and age were provided, 79% of the combined populations were male. The mean and median ages were 35 and 33 years old, respectively (range; 13-79). The mean (± SD), median, range of blood mitragynine concentrations and positivity rate for DUID and postmortem populations are shown in Table 1.

	DUID	Postmortem
Mean (±SD) (ng/mL)	141 (±177)	484 (±794)
Median (ng/mL)	81	140
Range (ng/mL)	<5.0–490	7.1–4200
% positive	0.13	0.59

Table 1. Blood concentrations and percent positivity for DUID and postmortem populations

Of six DUID mitragynine positive cases, opioids (oxycodone and tramadol) were concurrently reported in four cases. Additionally, benzodiazepines (clonazepam, clonazolam and etizolam) were reported in three of the four cases with opioids. A low concentration of methamphetamine (13ng/mL) and amphetamine at the therapeutic concentration (140ng/mL) were also reported in two of six cases.

In the postmortem subset (n=168), mitragynine was the only finding in six cases submitted from various states with a mean concentration of 1,160ng/mL (range; 220–3,700ng/mL). Four additional cases reported mitragynine in conjunction with midazolam (2), lidocaine and caffeine (1), and low ethanol (1). A majority showed polypharmacy; 96% were positive for at least 1 other drug; 38%, 20%, and 7.1% were positive for 3+, 5+ and 7+ drugs.

66% (n=111) were accompanied by other opioids: fentanyl (n=32), fentanyl analogs (n=7), heroin (n=5), combinations (n=40), and other opioids including methadone, oxycodone, tramadol, hydrocodone, and loperamide (n=27). Another drug class often observed in combination with mitragynine was CNS depressants in 44% (n=75); ethanol was most commonly detected (n=30), followed by benzodiazepines (n=12) and diphenhydramine (n=11). Cocaine, methamphetamine and amphetamine dominated the CNS stimulant class (28%; n=48), followed by cannabinoids (17%; n=29).

Conclusion/Discussion: Mitragynine is predominantly found in postmortem investigation cases, but its toxicity continues to be debated. Polypharmacy was commonly observed, particularly with opioids. The concentrations of mitragynine are important for toxicological interpretation and can aid the determination of the role mitragynine played as primary cause of death or a contributory factor.

Keywords: Mitragynine, Kratom, concentrations

Synthetic Cannabinoid Deaths in State of Florida Prisoners

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Background/Introduction: Synthetic cannabinoids (SCs), commonly referred to as K2 or spice, are potentially life-threatening novel psychoactive substances that are not routinely tested for in decedents. The potency and inconspicuous nature of SCs allows them to be smuggled into high security facilities, posing a threat to prison populations. Compounds in this drug class are commonly present as liquids or laced on plant material. "Spice paper," paper saturated with a solution containing SCs and dried, allows for delivery via the postal service or smuggling by visitors and employees. All the SCs encountered in this study are classified as Schedule I controlled substances according to a temporary scheduling order enacted by the Drug Enforcement Administration.

Objective: This research aims to report the details of SC fatalities in an at-risk population. Presenting the detected metabolites, case histories, concomitant drugs, and demographic data of these decedents is intended to increase awareness and prevent underreporting.

Method: Analysis for SCs was conducted on samples from 42 prisoner deaths submitted by State of Florida Medical Examiner Offices to the University of Florida Forensic Toxicology Laboratory. These deaths occurred over a 15-month period (March 2017 - May 2018). Urine samples from 41 prisoner deaths were sent to NMS Labs (Willow Grove, PA) for their Expanded Synthetic Cannabinoid Metabolites Screen using High Performance Liquid Chromatography/Tandem Mass Spectrometry (Analysis Code 9562U). The SC metabolites confirmed by this analysis were found to be: 5F-ADB 3,3-dimethyl-butanoic acid, FUB-AMB 3-methyl-butanoic acid, MDMB-FUBINACA 3,3-dimethyl-butanoic acid, and AB-CHMINACA 3-methyl-butanoic acid. These metabolites are referred to as their parent compounds (5F-ADB, FUB-AMB, MDMB-FUBINACA, and AB-CHMINACA) for the sake of brevity. One blood sample yielded a positive result for the parent compound 5F-ADB when urine was unavailable (Analysis Code 9560B).

Results: 5F-ADB (n=41, 97.6%) and FUB-AMB (n=15, 35.7%) were the most prevalent SCs in this population, while MDMB-FUBINACA (2.4%) and AB-CHMINACA (2.4%) were each detected in one prisoner. A combination of 5F-ADB and FUB-AMB was detected in 33.3% of cases (n=14). 5F-ADB was also present in the individual positive for MDMB-FUBINACA and the individual positive for AB-CHMINACA. Within this group, 90.5% (n=38) of the toxicology request forms indicated suspected SC use or a history of SC abuse upon submission to the forensic toxicology laboratory. Twenty-one cases (50.0%) had no other analytes detected in a comprehensive drug screen, ten (23.8%) have not completed testing, and eleven (26.2%) contained other analytes (acetaminophen, acetone, cannabinoids, carbamazepine, chlorpheniramine, desmethylsertraline, dicyclomine, diphenhydramine, ethanol, N-ethylpentylone, etomidate, hydromorphone, ketamine, lidocaine, morphine, normeperidine, norketamine, olanzapine, sertraline, ticlopidine, trimethoprim, venlafaxine). Five cases (11.9%) contained substances with significant abuse potential and six cases (14.3%) involved uncommonly abused medications. All the decedents in this report were male. The most common age range was between 40 and 49 years old (n=14, 33.3%) with a median age of 44. Twenty-three (54.8%) of the decedents identified as white and nineteen (45.2%) identified as black.

Conclusion/Discussion: The presence of SCs in overdose fatalities is underreported due to the lack of testing. Two reasons for this are, (1) SC analysis is costly; and (2) SC analytes evolve rapidly and require continuous monitoring. It is vital that medical examiners/coroners and forensic toxicologists recognize prisoners as a vulnerable population for SC overdoses to prevent oversights in their investigations. Additionally, this is a public health and safety issue that must be addressed with prevention programs and further research.

Keywords: Synthetic Cannabinoids, Prisoner Overdose, Postmortem Toxicology

PLATFORM

Not So Fast, My Friends: Toxicological Detection of the Synthetic Cannabinoids, 5F-ADB and FUB-AMB

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Introduction: Synthetic cannabinoids are implicated in intoxications and hospitalizations, as well as associated with impairment and cause of death. Two synthetic cannabinoids, 5F-ADB and FUB-AMB, emerged in the USA in 2015-2016. 5F-ADB's chemical name is methyl-2-{[1-(5-fluoropentyl)-1H-indazole-3-carbonyl]amino}-3,3-dimethylbutanoate and has a molecular weight 377.4 g/mol. FUB-AMB's chemical name is methyl-2-{[1-(4-fluorophenyl)methyl-1H-indazole-3-carbonyl]amino}-3-methylbutanoate and has a molecular weight 383.4 g/mol. Both drugs were first reported by the Drug Enforcement Administration (DEA) in 2015 and made Schedule I controlled substances in 2017. Since their emergence and eventual scheduling, both compounds have remained in the top five detected synthetic cannabinoid analytes in our toxicology casework. Both compounds are of the indazole-3-carboxamide family of structural variants and consist of an indazole core structure, a carboxamide linking group, and methyl ester side chains (a $_{L}$-*tert*-leucinamide moiety for 5F-ADB and a $_{L}$-validamide moiety for FUB-AMB). The methyl ester side chain has been shown to potentially be degraded by blood carboxylesterase enzymes into carboxylic acid metabolites.

Objective: In 2017-2018, we encountered a series of postmortem toxicology cases in which the subjects were purportedly witnessed to be smoking an herbal incense/potpourri product and found deceased at a later time. No synthetic cannabinoids were detected in routine toxicological testing for the parent drugs, yet materials acquired from the scene were identified as either containing 5F-ADB or FUB-AMB. Seeing as how the methyl ester side chain has the potential to be readily hydrolyzed via carboxylesterase enzymes, our objective was to develop and validate a method for the detection of the butanoic acid metabolites of 5F-ADB and FUB-AMB in blood.

PLATFORM

Method: An LC-MS/MS method was developed and validated for the quantification of the butanoic acid metabolites of both 5F-ADB and FUB-AMB in blood specimens. Extraction of the metabolites was completed via a protein precipitation with acetonitrile. Attributes assessed during validation were linearity, accuracy and precision, matrix selectivity, carryover, and exogenous drug interferences. After validation, authentic casework was selected for testing using the following criteria: 1) the case had circumstances involving 5F-ADB or FUB-AMB and was negative for both parent substances OR 2) the case was positive for parent 5F-ADB or FUB-AMB OR 3) the case had circumstances involving generic synthetic cannabinoids and was negative for all parent synthetic cannabinoids tested

Results: The linearity of the assay was 0.50-100 ng/mL. No carryover was detected in blank specimens injected after blood specimens spiked with 500 ng/mL analytes of interest. Accuracy and precision were within acceptable tolerances at two levels (4.00 and 40.0 ng/mL) and no exogenous drug interferences were documented. Eight sources of blood were tested during matrix selectivity assessment and acceptability criteria were met. All quantitative validation criteria were met.

Fifty six blood specimens were tested with this method. The 5F-ADB butanoic acid metabolite was detected in 28 specimens (range, 1.57-100 ng/mL; mean, 25.5 ng/mL). Of those 28 specimens, the parent drug was only detected in 7 instances. The FUB-AMB butanoic acid metabolite was detected in 10 specimens (range, 0.93-143 ng/mL; mean, 59.8 ng/mL). Of those 10 specimens, the parent drug was not detected. There were zero cases where the FUB-AMB parent drug was detected with metabolite.

Discussion/Conclusion: During 2017, 5F-ADB and FUB-AMB remained among the top detected synthetic cannabinoids by the DEA in seized drug evidence. In addition to testing for the parent drug, we developed a method for the determination of the butanoic acid metabolites of 5F-ADB and FUB-AMB in blood specimens. During toxicological testing in our laboratory, 5F-ADB or FUB-AMB metabolite was found on its own in 81.5% of cases that tested positive for either metabolite. These findings suggest that if only the parent drug is monitored in routine testing, the potential for false negative results is substantial.

Keywords: synthetic cannabinoids, 5F-ADB, FUB-AMB

S20

Keeping Austin Weird: Designer Drug Trends in Travis County, Texas

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Background/Introduction: Despite active government regulation of novel psychoactive substances (NPS), new compounds with unknown toxicities are continually emerging, resulting in adverse events. These purported "legal highs" pose problems for toxicologists who must identify an unending variety of new drugs of abuse. Their identification in biological matrices is essential to aid in interpretation of NPS results for both human performance and postmortem casework.

Objective: A retrospective analysis was conducted to characterize trends observed in NPS-positive postmortem cases from Travis County, Texas and surrounding counties in 2017. Interesting findings from select postmortem case reports will also be highlighted.

Method: All specimens analyzed were collected at autopsy at the Travis County Medical Examiner's Office. Postmortem femoral blood, vitreous, muscle and/or urine specimens were screened for volatiles utilizing headspace dual column gas chromatography (GC) with flame ionization detection (FID) and drugs of abuse by immunoassay (ELISA) for eight drug classes. Acid/neutral and alkaline qualitative drug screens were also performed by GC-mass spectrometry (MS) following liquid-liquid extraction procedures. Toxicological identification of NPS involved targeted confirmation using liquid chromatography tandem mass spectrometry (LC-MSMS), and/or non-targeted full-scan GC-MS.

Results: Of 1,744 toxicological cases analyzed from January through December 2017, 1.4% of cases (n=25) tested positive for one or more NPS. Synthetic opioids were the most common class of NPS detected (13 cases, 52%), followed by synthetic cannabinoids (12, 48%), designer benzodiazepines (2, 12%), and synthetic cathinones (2, 8%). Over the first six months of 2017, 5F-ADB was the most predominant finding (10, 40%), followed by furanyl fentanyl (4, 16%). The latter half of the year saw the emergence of cyclopropyl fentanyl (3, 12%) and methoxyacetyl fentanyl (2, 8%), as well as the synthetic cathinones, *N*-ethyl pentylone (2, 8%) and *N*-ethyl hexedrone (1, 4%). Additional psychoactive substances were detected in 84% (n=21) of NPS-positive cases; synthetic cannabinoids were often detected alone, or in combination with ethanol and THC, whereas synthetic opioids were typically found with additional NPS or CNS depressants. Heroin was only detected in 15% (n=2) of synthetic opioid cases. Decedent demographics were mostly male (23, 92%) and White (20, 80%), with mean and median ages (range) of 35.7 and 33.0 (19-68) years old, respectively. Postmortem cases encompassed drug toxicity and overdoses, accidental drownings, gunshot wounds, cardiovascular disease, and suicide via drug overdose. The manner of death for the majority of NPS cases was accidental (22, 88%), with the remaining three cases ruled as a suicide, homicide and natural death. NPS were directly related to cause of death in 86% (n=19) of accidental deaths.

Conclusion/Discussion: Based upon the toxicological analysis of postmortem cases in 2017, NPS were typically found in combination with other NPS, ethanol, benzodiazepines, cannabis and combinations thereof. Only four of 25 NPS-positive cases involved only one substance. Over the course of the year, a shift from synthetic cannabinoid-involved fatalities to synthetic opioid- and synthetic cathinone-involved fatalities was observed, with 5F-ADB being the most common NPS identified in postmortem casework. NPS were directly related to cause of death, or were contributory findings, in the majority of accidental fatalities. The continuous emergence of new NPS poses constant challenges for both clinical and forensic toxicologists. It is imperative that laboratories respond rapidly, adapt to changing NPS trends on the drug market, and improve analytical methodologies to improve the detection of these compounds.

Keywords: Novel psychoactive substances, postmortem toxicology, LC-MSMS

Deaths Related to Abuse of Methoxyacetylfentanyl and Cyclopropylfentanyl in the United States

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Background/Introduction:

Illicit fentanyl and fentanyl analogs have been found in contaminated or substituted heroin and counterfeit pill formulations since 2013 and 2014, respectively. Fentanyl analog trends began with the appearance of acetylfentanyl in 2013, followed by butyrylfentanyl in 2014. Starting in late 2015, the number of new fentanyl analogs began to proliferate, among the most prevalent were furanylfentanyl, carfentanil, acrylfentanyl, and para-fluoroisobutyrylfentanyl (FIBF), with smaller numbers of cases involving 3-methylfentanyl, tetrahydrofuranylfentanyl (THFF) and valerylfentanyl. In late 2017, increases in rates of detection of methoxyacetylfentanyl and cyclopropylfentanyl were observed and there are indications that they are among the most prominent of the fentanyl analogs contributing to fatalities currently.

Objective: The purpose of this presentation is to present and discuss the concentrations of methoxyacetylfentanyl and cyclopropylfentanyl detected in post-mortem whole blood samples, and the case histories for all cases.

Method: Analysis was performed using a Waters Xevo TQ-S Micro tandem mass spectrometer coupled with a Waters Acquity UPLC® (Milford, MA). Chromatographic separation was achieved using an Agilent Poroshell EC C-18 column (3.0 mm x 150 mm, 2.7 um) (Santa Clara, CA) heated to 60°C with a flow rate of 0.5 mL/min. The mobile phases used for analysis were 5 mM ammonium formate (pH=3) and 0.1% formic acid in methanol.

Blood samples (0.5 mL) were extracted using solid phase extraction (SPE) with 130 mg Clean Screen® DAU extraction columns. SPE columns were conditioned and washed before eluting with ethyl acetate/acetonitrile/ammonium hydroxide. Samples were evaporated to dryness and reconstituted in mobile phase.

Results: Blood samples from 42 cases (11 from Florida, 12 from Tennessee, 8 from Michigan, 11 from Illinois) involving cyclopropylfentanyl or methoxyacetylfentanyl were submitted for quantitative analysis. The deaths occurred between June and August 2017. Subject age was provided in 37 of the 42 cases and the mean (±SD) age was 41 (±11), with a median age of 43. Thirty-five individuals were male and 7 were female. The mean (±SD) and median concentrations for 14 cases testing positive for methoxyacetylfentanyl were 17.7 (±11.5) ng/mL and 15.1 ng/mL, respectively, with a range of 0.21-39.9 ng/mL. The mean (±SD) and median concentrations for 30 cases testing positive for cyclopropylfentanyl were 15.2 (±11.9) ng/mL and 12.3 ng/mL respectively, with a range of 1.4-43.3 ng/mL.

Only two of the 41 cases we analyzed had no other significant toxicology besides the findings of 12 and 20 ng/mL of methoxyacetylfentanyl, close to the median concentrations across all cases (15.2 ng/mL). Other fentanyl derivatives detected in these cases in addition to methoxyacetylfentanyl and cyclopropylfentanyl included acrylfentanyl (n=2), FIBF (n=2), furanylfentanyl (n=1), and acetylfentanyl (n=1); in addition to U-47700 in 2 cases, a non-fentanyl related novel illicit opioid initially encountered in 2015.

Additional common drugs of abuse found in these cases were: Cocaine and/or its metabolite benzoylecgonine (BZE) in 21 cases, morphine was detected in 17 cases, and benzodiazepines in 12 cases. Concurrent detections of morphine, cocaine, and benzodiazepines have been established as common findings with illicit fentanyl.

Conclusion/Discussion: There are no previously published data to assist with the interpretation of cyclopropylfentanyl or methoxyacetylfentanyl concentrations or findings, so these cases have to be evaluated carefully based on their merits and on the basis of thorough scene investigation and medical history, along with findings from a full autopsy, as the diagnosis becomes one of exclusion. Methoxyacetylfentanyl and cyclopropylfentanyl are among the latest fentanyl analogs to emerge with some prevalence onto the opioid drug market, potentially being sold to unsuspecting users as a contaminant in, or substitute for, heroin and/or other recreational opioids. These compounds have not previously been studied or described in humans; therefore, pharmacological effects and toxicity can only be inferred from case reports.

Keywords: Cyclopropylfentanyl, Methoxyacetylfentanyl, Post-Mortem

PLATFORM

Prevalence of Cocaine and its Metabolites in Postmortem Specimens Submitted for Toxicological Investigation in New York City in 2017

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Background/Introduction: Cocaine is a popular recreational drug and potent central nervous system stimulant. Analysis of cocaine and its metabolites in routine biological specimens presents little challenge to modern forensic toxicology laboratories equipped with GCMS and/or LCMS capability. However, detection of the parent drug, cocaine, in cases of suspected cocaine-related death is dependent on a number of factors including the dose consumed, individual metabolism, hydrolysis after death and the presence or absence of a preservative. Decomposition processes further hinder the detection of cocaine in blood compromising the assessment of recent use of the drug prior to death. Where blood is not available, alternative postmortem specimens that can help determine recent use are preferred but definitive research is limited on what the most appropriate specimen would be for this assessment. Vitreous humor and brain are two of the most commonly evaluated as an alternative but the majority of previous studies were based on small sample sizes and did not provide a conclusive result.

Objective: The objective of this study is to describe the prevalence of cocaine and cocaine metabolite distribution in all postmortem forensic toxicology cases within New York City between January 1 and December 31 2017.

Method: In 2017, 5,342 postmortem cases were submitted for toxicological investigation by the Medical Examiners from all five boroughs of New York City. Toxicology reports with any detectable cocaine or cocaine metabolite, most commonly benzoylecgonine (BE), ethylbenzoylecgonine (EBE) and ecgonine methyl ester (EME), from January 1 to December 31 2017 were retrospectively analyzed. Information regarding decedent demographics and all drugs detected and/or quantified were recorded.

Results: A total of 937 decedents (17.5% of submitted cases) were found with detectable levels of cocaine or one of its metabolites and 1846 individual specimen samples were included in the analysis. Of the 937 decedents, 739 (79%) were male, with an average age of 46 years at the time of death. The majority of decedents were identified as Black (38%), followed by White (30%), and Hispanic (30%). Of the 937 decedents, 926 (99%) had detectable BE, 271 (29%) EBE, 245 (26%) cocaine, and EME, 23 (2%). Of the 1846 samples, the most common samples tested were femoral blood (636; 34%), vitreous humor (441; 24%), urine (228; 12%), cardiovascular blood (157; 9%), hospital blood (75; 4%), and brain (69; 4%). 50 (3%) samples of decomposition fluid were also included in the analysis. Finally, of the 765 decedents with confirmed manner/ cause of death information, 685 (90%) had an accidental substance-related overdose as the primary cause of death and 642 (84%) had cocaine as a contributing factor listed on the death certificate.

Conclusion/Discussion: This is the first study to describe the prevalence of cocaine and its metabolites amongst decedents in New York City. The large sample size (937) of decedents warrants additional testing of available vitreous humor and brain samples, to compare concentrations between different biological samples, and between accidental versus natural deaths. This comparison will elucidate the most reliable tissue sample in determining cocaine toxicity in postmortem cases, especially when cocaine is not present in blood samples.

Keywords: Cocaine & metabolites, intoxication, postmortem

PLATFORM

Screening of Fentanyl Analogs in Whole Blood using LC-QTOF Analysis

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Background/Introduction: Recently, synthetic analogs of fentanyl (up to 10,000 times more potent than morphine) have been introduced into the illicit drug market as cutting agents for heroin. As a result, the opioid epidemic has grown in severity and the number of fatal overdoses has increased significantly. Fentanyl analogs are often difficult to detect in biological samples due to their low concentrations and similar molecular structures.

Objective: The goal of this project was to develop and validate a comprehensive screening method for 14 fentanyl analogs in whole blood using liquid chromatography-quadrupole-time-of-flight mass spectrometry (LC-QTOF) according to SWGTOX guidelines. For verification of applicability, the method was then applied to an authentic post mortem case. The compounds of interest for this assay were: fentanyl, alfentanil, acetyl fentanyl, butyryl fentanyl, remifentanil, carfentanil, cis-3-methylfentanyl, 4-ANPP, furanyl fentanyl, isobutyryl fentanyl, norcarfentanil, valeryl fentanyl, norfentanyl, and sufentanil

Method: Whole blood samples (0.5 mL) were fortified with methanolic standards containing 14 fentanyl analogs and deuterated internal standards. Samples were subjected to an optimized solid-phase extraction procedure utilizing a polymeric column and compounds were eluted with 5% ammonium hydroxide in ethyl acetate. Analyses were performed using an Agilent Technologies 1290 Infinity liquid chromatograph coupled to an Agilent Technologies 6530 Accurate Mass Time-of-Flight mass spectrometer (LC-QTOF). Data were acquired in both TOF mode and All Ions Fragmentation (AIF) mode to serve as presumptive and confirmatory identifications, respectively. A Personal Compounds and Database Library (PCDL) was produced in-house containing all analytes of interest. Additional expanded PCDLs were created for other drugs of abuse. The assay was validated according to the Scientific Working Group for Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology guidelines. Parameters assessed were matrix effects, interferences, stability, and limit of detection. For proof of applicability, central (heart) and peripheral (femoral) blood from a suspected fentanyl fatality were analyzed using the optimized and validated screening method.

Results: The limit of detection for all analytes was 0.1-0.25 ng/mL and 0.1-1.0 ng/mL in TOF and AIF modes, respectively. No carryover or interferences (exogenous or endogenous) were observed. Matrix effects were considered acceptable for most analytes with minor ion enhancement values ranging from 1-14.4%. Matrix effects for the nor- analytes (metabolites) exhibited higher ion suppression values of 28-37%; however, the effects were reproducible based on %CV and compensated for with a suitable internal standard. Stability was assessed after 24 hours in the autosampler (22°C) and the refrigerator (4°C) and was determined to be acceptable with ±5 and ±18% bias, respectively. The central and peripheral blood samples screened positive for morphine, codeine, methamphetamine, 6-monoacetylmorphine, fentanyl, alprazolam and etizolam using both data acquisition modes. Analytes outside of the scope of this assay were verified chromatographically and spectrally with reference standards. The peak areas for all opioids in the central blood were approximately three times greater than the peripheral blood; suggesting postmortem redistribution. More specifically, the response of fentanyl in the blood samples fell between the responses of the high and low QCs (5 and 1 ng/mL, respectively), thus providing presumptive information on drug concentration in the sample. The blood samples also screened positive for 4-ANPP (central) and cocaine (central and peripheral) using TOF mode only, which were previously undetected by the initial laboratory.

Conclusion/Discussion: This research presents a validated and comprehensive method for the screening of fentanyl analogs in whole blood using LC-QTOF analysis with low limits of detection. In addition, the method was successfully applied to the analysis of a postmortem case containing fentanyl analogs and other drugs of abuse.

Keywords: Fentanyl analogs, Postmortem, LC-QTOF

PLATFORM

S24

An Efficient Screening Approach for Fentanyl Analogs Using A Single Extraction Sequential GC/MS and LC/MS/MS Analysis

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Background/Introduction: All suspected overdose cases admitted to the Franklin County Coroner's Office Forensic Toxicology Laboratory have required dedicated, labor intensive, replicate analysis to screen and confirm fentanyl analogs by LC/MS/MS. A duplicate extraction approach requires more biological sample to be consumed and delays case completion. Compounding this is the ever changing array of fentanyl analogs with concentrations appearing in blood at less than 5 ng/mL, making traditional GC/MS full-scan detection and immunoassay screens ineffective.

Objective: To describe an efficient and adaptable single extraction sequential (SES) GC/MS and LC/MS/MS protocol enabling fentanyl analog screening from routine and previously analyzed blood alkaline drug concentrates. Fentanyl analogs are typically undetectable using GC/MS full-scan detection alone or fall short of recommended forensic identification criteria without duplicate LC/MS/MS extractions. The following protocol allows for sample conservation, overall testing efficiency, and the best of both GC/MS and LC/MS/MS detection capabilities.

Method: All cases submitted for medico-legal toxicology investigation receive a basic-drug solid phase extraction and GC/MS full-scan analysis on 1 mL of blood fortified with methapyrilene (internal standard). To accommodate the sequential LC/MS/MS analysis, fentanyl-d5 and norfentanyl-d5 internal standards are added to the blood samples at concentrations not visible in the full-scan GC/MS analysis. A negative and fentanyl analog fortified positive control are extracted with each batch. After the alkaline GC/MS full-scan analysis is complete, all non-trauma deaths, including potential overdoses, naturals, and SUID case extracts are separated with the positive and negative fentanyl analog controls. By directly adding LC mobile phase (acidified water and ACN), to these extracts, fentanyl analog screening by LC/MS/MS can be performed as a sequential extension of the routine basic compound analysis. The low concentration fentanyl internal standards and analogs are easily visible by the LC/MS/MS using a targeted SRM mode, down to approximately 100 pg/mL. All presumptive positive fentanyl analog cases are then ordered for subsequent extraction and LC/MS/MS confirmation. SWGTOX LOD, stability, interference, and suppression/enhancement studies for qualitative screens were evaluated and demonstrated acceptable results for the following compounds of interest: fentanyl, norfentanyl, alfentanyl, sufentanyl, carfentanil, acrylfentanyl, furanylfentanyl, 3-methylfentanyl, 3-fluorofentanyl, fluorobutyrylfentanyl, fluoroisobutyrylfentanyl, acetylfentanyl, valerylfentanyl, cyclopropylfentanyl, methoxyacetylfentanyl, beta-hydroxy thiofentanyl, ocfentanil, tetrahydrofuran (THF) fentanyl, butyrylfentanyl, phenylfentanyl, U-47700, U-49900, and AH-7921.

Results: This tandem instrument protocol allows for efficient dual analysis of the same sample extract (broad scope basic drug GC/MS analysis and targeted LC/MS/MS screening) to detect low concentration fentanyl analogs, while conserving evidentiary biological samples, and satisfying the preliminary screen analysis. The LC/MS/MS analog detection method can be expanded or modified to include data dependent fragmentation, MRM, or alternate analysis of the extract by an analytical platform such as TOF-LC/MS, depending on the laboratory's available instrumentation.

Conclusion/Discussion: In post-mortem toxicology, ensuring a full systematic toxicological approach can be performed on a finite sample volume is a frequent challenge and limited hospital admission fluids typically exacerbate this issue. Additionally, while immunoassay detection is a common screening tool, a la cart immunoassay kit development and sensitivity for each analog may be difficult for manufacturers and not feasible for laboratory testing long term. This single extraction sequential (SES) instrument analysis can be used as an expansion of basic drug GC/MS analysis routinely performed by most forensic toxicology labs. This method is easily adaptable to any LC/MS/MS detection platform to ensure progressive detection of designer analogs in the future. This thorough testing approach enables the Franklin County Coroner's Office Forensic Toxicology Laboratory to conserve labor and resources while screening for low concentration compounds that may otherwise go undetected.

Keywords: Fentanyl, Analogs, Screen

Rapid determination of cocaine and its metabolites in serum using differential ion mobility tandem mass spectrometry (DMS-MS/MS)

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Background/Introduction: Cocaine is a potent central nervous system stimulant that blocks the reuptake of dopamine, serotonin, and norepinephrine resulting in a state of euphoria and increased alertness. It continues to be one of the most abused illicit drugs in the United States. Rapid methods are needed for the identification and quantitation of cocaine and its major metabolites, benzoylecgonine (BE), ecgonine methyl ester (EME), and cocaethylene (CE), in biological specimens by clinical and forensic toxicology laboratories. Differential ion mobility spectrometry (DMS) is a technique for post-ionization differentiation that separates ions in the gas-phase based on the ions' mobility in high and low electric fields. DMS-based separation methods combined with mass spectrometry are effective at the rapid separation of ions at atmospheric pressure and have been shown to improve the limitations with chromatography-based methods.

Objective: To develop and validate a differential ion mobility spectrometry tandem mass spectrometry (DMS-MS/MS) method for the analysis of cocaine and its major metabolites in human serum that requires minimal sample preparation and no column chromatography.

Method: A Shimadzu Nexera X2 ultrahigh performance liquid chromatography system (UHPLC) was used to infuse the samples directly into the DMS cell at a rate of 30 µL/min. Separation of cocaine and its metabolites in serum were performed in a SelexION DMS component from Sciex coupled to a QTRAP 6500 with an IonDrive Turbo V source for TurbolonSpray® using acetonitrile as a chemical modifier. Compensation voltage was ramped from -35 V to -6 V over 1 min while monitoring the MRM transitions of each analyte. Sample batches were analyzed for linearity, bias, imprecision, carryover at the highest calibrator (1,000 ng/mL), and endogenous and exogenous interferences. The calibration range for cocaine and its metabolites was 10-1,000 ng/mL. The limit of quantitation was set at 10 ng/mL. Quality control specimens were 30 ng/mL (Low QC), 300 ng/mL (Mid QC), and 750 ng/mL (High QC). Cocaine and/or BE positive human serum samples (n=33) previously analyzed by UHPLC-MS/MS were quantified by the new DMS-MS/MS method and evaluated for variability.

Results: The linear regression coefficients of determination (r^2) of 0.9912 or greater was determined for each analyte. The intra-day and inter-day imprecision were < 20% CV for all controls (n=3/batch, 5 batches). No carryover or interferences were detected. Variability between the two methods was 30% or less for cocaine and BE. EME was found in concentrations ≤ 10 ng/mL, and CE was not detected in any of the samples.

Conclusion/Discussion: Presented is a method using minimal sample preparation and DMS-MS/MS to quantitate cocaine and its metabolites in human serum. DMS-based separation methods combined with mass spectrometry can provide a viable platform for high throughput analysis for a variety of drugs of abuse and their metabolites commonly analyzed by clinical and forensic toxicology laboratories.

Acknowledgements: This work was supported by National Institute on Health [P30DA033934] and National Institute on Drug Abuse [T32DA007027-42].

Keywords: Cocaine, differential mobility spectrometry, DMS-MS/MS

S26

Simultaneous Quantitation of Seven Synthetic Cathinones in Post-Mortem Samples by UHPLC-MS/MS

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Background/Introduction: Over the years, Miami-Dade County, FL has been at the forefront of the ebb and flow of designer drug trends. Even though "bath salts" are no longer in the evening news, synthetic cathinones have continued to appear in routine post-mortem casework for years. In general, they exhibit powerful stimulant effects and are linked to a number of homicides, suicides, accidents, and overdoses. Since 2015 there have been more than 300 cases of this drug class detected at the Miami-Dade County Medical Examiner Department.

Objective: The objective of this study was to develop and validate a method to simultaneously quantify methylone, ethylone, butylone, dibutylone, pentylone, alpha-pyrrolidinopentiophenone (α-PVP), and n-ethylpentylone (ephylone) in post-mortem blood, serum, and tissue at the Miami-Dade Medical Examiner. The quantitative data obtained will then be used to better understand what role synthetic cathinones play in relation to cause and manner of death.

Methods: A method validation was conducted in accordance with the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines. This included assessment of linearity, extraction recovery, precision, bias, dilution integrity, carryover, sample stability, and exogenous interferences. Samples were fortified with a deuterated internal standard for each analyte and a buffer before solid phase extraction using mixed-mode ion exchange columns. The eluent was dried down and reconstituted in mobile phase, then analyzed with a Shimadzu Nexera X2 Ultra High-Performance Liquid Chromatograph coupled to a Shimadzu 8060 Tandem Mass Spectrometer in MRM mode. Separation was achieved on a Restek Raptor ARC-C18 column at a flow rate of 0.8 mL/min.

Results: Baseline resolution was achieved for all analytes in under six minutes. Analyte linearity was established from 10-500 ng/mL on a six point calibration curve with a limit of detection administratively set at 5 ng/mL. Extraction recovery was 95-100% for all analytes. Inter-day and intra-day coefficient of variation was ≤ 3.2% and bias ranged from -6.8-0.8% deviation from target value. Dilution integrity was evaluated on 1:1, 1:4, and 1:9 dilutions and resulted in no significant impact to precision or bias. No carryover was detected in an extracted matrix blank after an injection at 1000 ng/mL and all analytes remained stable in the autosampler for 72 hours. No interferences were observed from other commonly encountered drugs and metabolites.

Conclusion/Discussion: A comprehensive UHPLC-MS/MS method was successfully validated for the most common synthetic cathinones seen in Miami-Dade County, FL. The application of this method will bring more data points for methylone, ethylone, butylone, dibutylone, pentylone, α-PVP, and n-ethylpentylone to the scientific world, so that the role of these drugs can be better understood for medical examiners and toxicologists alike.

Keywords: Cathinones, N-ethylpentylone, UHPLC-MS/MS

S27

Quantitation and Identification of 370+ Synthetic Cannabinoids in Blood using Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF/MS)

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Background/Introduction: Synthetic cannabinoids, also known as synthetic marijuana or a number of common names such as Spice, Kush, or K2, have been implicated in overdoses and fatalities nationwide. These drugs are designed to interact with the body's cannabinoid receptors but are far more potent than traditional marijuana and cause harmful side effects. Users under the influence of these compounds exhibit behaviors ranging from hallucination, aggression, to paranoia. The Drug Enforcement Administration reports 922 identifications of synthetic cannabinoids in the 2017 Emerging Threat Report. The number of synthetic cannabinoids in forensic casework samples is expected to increase. However, many of these compounds are not being reported due to the inability of lab analyses and legislation to keep up with the creativity of the underground labs who can produce new compounds with a change in structural chemistry.

Objective: To validate a comprehensive method in accordance with SWGTOX Guidelines capable of identifying and quantifying an extensive number of synthetic cannabinoids and their metabolites in blood using the LC-QTOF/MS.

Method: Using the novel Synthetic Cannabinoids Screening Library plates from Cayman Chemical (Ann Arbor, MI), we validated a method and created a Personal Compound Database and Library (PCDL) complete with retention times for 370+ synthetic cannabinoids. Validation parameters evaluated included limit of detection (LOD), limit of quantitation (LOQ), linearity, sample stability, carryover, interference, and matrix effects (ion suppression). Blood samples were subjected to an ethyl acetate/hexane liquid-liquid extraction. Samples were then evaluated using an Agilent 1260 Infinity Liquid Chromatography system coupled with a 6530 Accurate Mass Quadrupole Time-of-Flight Mass Spectrometer. Agilent MassHunter Qualitative Data Analysis, Quantitative Data Analysis, and PCDL were utilized to identify compounds.

Results: The 370+ Synthetic cannabinoids were divided into the following groups for ease of reporting:

1 Benzoylindoles, 2 Dibenzopyrans, 3 Indazole-3-carboxamides, 4 Naphthoylindoles, 5 Phenylacetylindoles, 6 Tetramethylcyclopropanoylindoles, and 7 *Cyclohexylphenols*. Examples from each Synthetic Cannabinoid (SC) Group are reported below:

SC Group	Representative Compounds	LOD (ng/mL)	LOQ (ng/mL)	Bias at LOQ (%)	Within-Run CV at LOQ (%)	Between-Run CV at LOQ (%)
1	RCS-4	0.8	4.0	-1.00	6.3	10.9
2	HU-210	4.8	9.6	6.21	11.0	18.9
3	AB-FUBINACA	0.9	4.6	-0.90	6.8	8.4
3	MAB-CHMI-NACA	0.9	4.6	5.02	5.9	6.2
4	JWH-018	0.9	4.2	1.22	5.1	11.7
4	JWH-073	0.8	4.0	-0.50	7.3	8.1
5	JWH-167	0.8	3.8	13.85	8.8	6.7
6	UR-144	0.8	3.8	-0.05	8.8	10.7
6	XLR-11	0.8	4.1	2.91	7.6	12.2

Cyclohexylphenols (ex. CP 47, 497) did not perform well with this method. These compounds are difficult to ionize attributed to the lack of nitrogen. Linearity was determined for each compound as weighted linear or weighted quadratic fit across a dynamic range of interest of ~ 0.5 ng/mL-50 ng/mL. Carryover was not detected in samples up to 1,000 ng/mL. Interferences between synthetic cannabinoids were evaluated and noted appropriately; for example, JWH 201 and JWH 302 are unable to be differentiated as they are methoxy positional isomers of each other (para- and meta- respectively).

Conclusion/Discussion: A comprehensive method using LC-QTOF/MS for the confirmation of an extensive number of synthetic cannabinoids and their metabolites in blood was successfully developed and validated. Although SWGTOX guidelines do not specifically address LC-QTOF/MS, the guidelines were adhered to as strictly as possible. This method is able to reliably identify and quantify synthetic cannabinoids at low concentrations and continues to evaluate samples against the largest library of synthetic cannabinoids available.

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

Quantification of Seven Novel Synthetic Opioids in Blood Using LC-MS/MS

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Background/Introduction: Opioid involved fatal overdoses constituted 66.4% of fatal overdoses that occurred in the United States in 2016, according to the Centers for Disease Control and Prevention (CDC). The CDC also reported that the number of deaths attributed to synthetic opioids other than methadone more than doubled from 2015 to 2016. Fatal and nonfatal overdoses were reported which involved the use of novel synthetic opioids (NSO) such as U-47700, AH-7921, U-49900, and MT-45 in the U.S. and Europe. There are a limited number of analytical methods published for the detection of U-47700, AH-7921, U-49900, U-50488, MT-45, W-18, and/or W-15.

Objective: The primary objective of this study was to develop, optimize, and validate a comprehensive method for the quantitation of seven synthetic opioids (U-47700, AH-7921, U-49900, U-50488, MT-45, W-18, and W-15) in whole blood using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Method: The blood samples (0.5 mL) were fortified with 25 µL of mixed methanolic calibrator or quality control solution and 10 µL of internal standard solution (U-47700-d_6, MT-45-d_{11}, and W-18-d_4 at 1,000 ng/mL in methanol). The analytes were isolated via a solid-phase extraction protocol. The optimized extraction method utilized a Cerex® Clin II (3 mL, 35 mg) SPE cartridge. Acidic and neutral compounds were eluted with ethyl acetate and alkaline compounds were eluted with 80:20:5 dichloromethane:isopropanol:ammonium hydroxide. The combined elutions were dried under nitrogen at 50 °C, and reconstituted in 1 mL of the mobile phase starting gradient. Analysis was performed using an Agilent 1290 Infinity II liquid chromatograph coupled to an Agilent 6470 triple quadrupole mass spectrometer. The injection volume was 5 µL. Analytes were separated using gradient elution on an Agilent Poroshell 120 EC-C18 column (100 x 2.1 mm, 2.7 µm) with 0.05% formic acid with 5 mM ammonium formate in water (A) and 0.1% formic acid in acetonitrile (B) at 0.5 mL/min. Data were acquired using dynamic multiple reaction monitoring with electrospray ionization in positive mode. The method was validated in accordance with Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology.

Results: Collection of both the acidic/neutral and alkaline elution fractions was necessary in order to isolate all seven analytes, as W-18 and W-15 eluted in the acidic/neutral fraction. The lower limits of quantification were 0.25 ng/mL for all analytes except W-18 (1 ng/mL) and the upper limits of quantification were 100 ng/mL for all analytes evaluated. The coefficients of determination (R^2) for the calibration curves were >0.99. Analytical bias, within-run precision, and between-run precision for three quality control concentrations were within ±15%, ≤16%, and ≤17%, respectively. Matrix effects were within ±19% and compensated for with matched deuterated internal standards when commercially available. All analytes were found to be stable in blood at room temperature for 24 h, 4°C for 72 h, and in the autosampler for 72 h. There was no carryover observed in the final method.

Conclusion/Discussion: A sensitive quantification method for the synthetic opioids U-47700, AH-7921, U-49900, U-50488, MT-45, W-18, and W-15 in whole blood was developed and validated in accordance with SWGTOX method validation guidelines. To our knowledge, this is the first reported comprehensive quantification method for these synthetic opioids in blood and the first method to successfully quantify W-18 and W-15 in blood. This method is applicable to driving under the influence (DUI) and fatal drug intoxication cases.

Keywords: Novel synthetic opioids, method validation, LC-MS/MS

Side-by-Side Comparison of LC-Q/TOF-MS and LC-MS/MS Validation Parameters for the Detection of Suvorexant in Blood

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Background/Introduction: Endogenous interferences and matrix effects are an important consideration in liquid chromatography-mass spectrometry (LC-MS) based assays. Using electrospray ionization (ESI), competition from the matrix or other analytes can decrease the efficiency of ionization in the source. This has the potential to produce significant downstream bias during quantitative analyses. Although the use of isotopically labelled internal standards that coelute with the drug of interest can mitigate this issue, rigorous method development, optimization, and validation is critical. Complex matrices, ineffective sample clean-up, disproportionate amounts of analyte, and short run times (coeluting species) increase the likelihood that these issues will arise. In this study, the potential for matrix effects and interferences were directly compared using liquid chromatography-quadrupole/time-of-flight-mass spectrometry (LC-Q/TOF-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Objective: To directly compare ion suppression/enhancement and the potential for endogenous and drug-related interferences using LC-Q/TOF-MS LC-MS/MS.

PLATFORM

Method: An Agilent Technologies 6530 Accurate-Mass LC-Q/TOF-MS and Agilent 6470 Triple Quadrupole Mass Spectrometer were used to detect suvorexant in whole blood following liquid-liquid extraction. The analytical method was previously validated using LC-Q/TOF-MS. Due to the absence of isotopically labelled suvorexant, estazolam-D5 was used as the internal standard. Ion suppression/enhancement was assessed qualitatively and quantitatively using post-column infusion and post-extraction addition techniques in accordance with recommended guidelines. Endogenous interferences from blood were evaluated and various commercial clean-up cartridges were used to further reduce phospholipids and other species. Interferences from common drugs were also evaluated qualitatively and quantitatively. Data obtained using LC-Q/TOF-MS and LC-MS/MS were compared in parallel and differences in performance are discussed.

Results: A side-by-side comparison of LC-Q/TOF-MS and LC-MS/MS validation data using a previously published (LC-Q/TOF-MS) method revealed interesting results. Using post-column infusion, no qualitative interferences were identified using LC-Q/TOF-MS, but significant ion suppression was observed using LC-MS/MS. This was attributed to late-eluting phospholipid interferences which were identified using gas chromatography-mass spectrometry (GC-MS). Matrix effects using post-extraction addition were determined to exceed acceptable limits using LC-MS/MS at low and high suvorexant concentrations (-35% and -26%, respectively) but were within acceptable ranges using LC-Q/TOF-MS method (16% and 15%). Proprietary cartridge-based devices including Agilent Captiva were evaluated to mitigate these effects. Although no qualitative interferences from common drugs were identified using either LC-MS platform, quantitative interferences due to decreased ionization efficiency were observed when coeluting species were present in concentrations in excess of the internal standard. The evaluation of qualitative interferences alone would not have identified these issues.

Conclusion/Discussion: These results highlight the need to critically evaluate interferences both qualitatively and quantitatively, particularly if isotopically labelled internal standards are not available. The potential for coeluting species to decrease overall ionization efficiency due to capacity-limited source ionization is demonstrated in this study. Systematic bias caused by reduced ionization efficiency in the electrospray source is not evident from peak shape, ion ratios or retention times, all of which may be within acceptable ranges. This may be problematic for fast LC methods where multiple drugs are simultaneously quantified. Moreover, as the proportion of interfering drug to target analyte become more disproportionate, the potential for bias is increased. Matrix and drug interferences can be somewhat mitigated by avoiding fast LC methods, using minimal specimen, injection volume, and selective sample clean-up steps. The potential for interferences is particularly important if isotopically labelled internal standards are not available. Moreover, differences in performance between LC-Q/TOF and LC-MS/MS-based assays should also be considered.

Keywords: Ion suppression, interferences, LC-Q/TOF-MS, LC-MS/MS

Quantification of U-47700 and its Metabolites in Plasma by LC-MS/MS

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Background/Introduction: Novel Synthetic Opioids (NSO) have played a major role in the US opioid epidemic. These substances gained popularity in the illicit drug market and include fentanyl derivatives, as well as other chemically unrelated opioid agonists. U-47700, a non-fentanyl analog analgesic opioid, was first developed by The Upjohn Company with a reported potency 7.5 times that of morphine. Like many NSO, U-47700 is sold as a research chemical that can be purchased online but can also be found in "Gray Death" a mixture of fentanyl(s), heroin, and U-47700. In order to identify recent use, several metabolites have been identified in vivo and in vitro for U-47700, including N-desmethyl-U-47700 (dm-U4) and N,N-didesmethyl-U-47700 (ddm-U4). However, there are no methods available in literature for quantification of U-47700 metabolites in biological specimens.

Objective: With the emergence of NSO, there is a need for laboratories to be able to detect these drugs and their metabolites in various matrices. In this study, a liquid chromatography tandem mass spectrometry (LC-MS/MS) method was optimized and fully validated to detect and quantify U-47700 and its metabolites, dm-U4 and ddm-U4, in human plasma.

Method: U-47700 and its metabolites were extracted from human plasma (100 µL) using an optimized solid phase extraction procedure. Briefly, plasma was fortified with calibrator or control solution and internal standard as well as phosphate buffer (900 µL). Following two aqueous and three organic washes, analytes were eluted with 80:20 dochloromethane:isopropanol with 5% ammonium hydroxide. Analysis was performed on an Agilent 1290 Infinity Liquid Chromatograph coupled to an Agilent 6470 Triple Quadrupole Mass spectrometer (Santa Clara, CA). Analyte separation occurred across an Agilent ZORBAX Eclipse Plus C18 column (1.8 µm, 2.1x50 mm) with matching guard (1.8 µm, 2.1x5mm). Separation was achieved with isocratic elution with 60:40 mobile phase A:B at 0.4 mL/min. Mobile phase A was 5 mM ammonium formate with 0.05% formic acid in deionized water. Mobile phase B was 0.1% formic acid in methanol. Electrospray ionization was operated in positive mode. A multiple reaction monitoring (MRM) method was used to detect the analytes with one transition for quantification and one for qualification. The method was validated according to SWGTOX guidelines, including: precision and bias, linearity, carryover, interferences, matrix effects, limit of detection (LOD), limit of quantification (LOQ), dilution integrity, and stability.

Results: LOD were 0.05ng/mL for U-47700 and dm-U4, and 0.1 ng/mL for ddm-U4. Linear ranges for U-47700 and dm-U4 were 0.1-100 ng/mL, while ddm-U4 quantified 0.5-100 ng/mL (R^2 >0.99). Matrix effects were assessed via post-extraction addition and were 95-105%, indicating little ion suppression or enhancement. Extraction recovery was >79%. Stability was evaluated in plasma at room temperature (24 h, 20°C), refrigerated (72 h, 4°C), and after three freeze/thaw cycles (-20°C) and after processing in the autosampler (72 hours, 4°C). Analytes were considered stable with <±10.6% change from baseline. Endogenous and exogenous interferences were evaluated and had no significant impact.

Conclusion/Discussion: This method was optimized and fully validated for quantification of U-47700 and its metabolites in human plasma. This is the first method, to our knowledge, which quantifies two phase I metabolites of U-47700: N-desmethyl-U-47700 (dm-U4) and N,N-didesmethyl-U-47700 (ddm-U4). The final, optimized method was more sensitive than previous studies and allows for comprehensive identification and quantification of primary metabolites. The limits of quantification are sufficiently low to detect intoxicating or fatal concentrations of U-47700. The method will be applied to analysis of rat plasma following controlled intravenous U-47700 administration.

Keywords: U-47700, Novel Synthetic Opioids, LC-MS/MS

Nitrous Oxide in Postmortem and DUID cases from 2013–2018

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Background/Introduction: Nitrous oxide is a colorless gas that is used the food industry in the production of whipped cream. It is also used in the medical field as "laughing gas" for dental procedures and emergency medicine due to its analgesic and anesthetic properties, and as a recreational inhalant drug because of its low cost, legality, ease of purchase, and short half-life. Nitrous oxide is sold in pressurized metal canisters that can be used to fill balloons from which users can inhale the gas. Other users dispense the nitrous oxide into a whipped cream dispenser called a "whippet" and inhale the gas from the nozzle. The gas is absorbed from the lungs into the bloodstream and, upon entering the brain, produces hallucinogenic and euphoric effects.

Objective: Concentrations of nitrous oxide in DUID and postmortem investigations between 2013 and 2018, the demographics of users, and case histories for both types of cases will be presented.

Method: Nitrous oxide was quantified on an Agilent 6890/5973 GC-MS system with an HP-Molesieve column (Agilent, Santa Clara, CA, USA). A 1 mL or 1 g sample with 10 mcL of carbon monoxide internal standard was prepared in a 20 mL headspace vial along with calibrators and controls. The headspace is sampled and analyzed using SIM acquisition after a 10-minute incubation at 70°C. The calibration range was 1.8–180 mcg/mL.

PLATFORM

Positive cases over a five-year period (January 2013-February 2018) were identified from the Laboratory Information Management System and the following information was extracted: gender, age, type of case (postmortem or DUID), reported concentration, number of days between collection and analysis, and additional test requests.

Results: Seventy positive cases were identified. Blood was analyzed in all cases and in two cases analysis of tissue was also performed. The average age was 32 years (range: 1–57 years). There were 4 DUID cases, 65 postmortem cases, and 3 cases of unknown origin. The concentrations detected for blood samples ranged from 2.29–273.5 mcg/mL; concentrations detected for lung samples were 2.65 mcg/g and 18.2 mcg/g. Concentration ranges were 2.29–8.01 mcg/mL and 2.54–273.5 mcg/mL for DUID and postmortem cases, respectively. Based on the information provided by the clients, the number of days between sample collection and extraction ranged from 3–54 days. Additional testing requested by clients included various screening and confirmation panels, alcohol, inhalants, DUID/DRE Toxicology, and Postmortem Toxicology. Half of the cases were nitrous oxide only cases and half were polydrug cases. Other compounds detected included cannabis, CNS stimulants, CNS depressants, narcotic analgesics, dissociative drugs, and others.

Case histories were available for one DUID and one postmortem case. The DUID case highlights two separate incidents involving hallucinations following nitrous oxide inhalation while driving. The postmortem case highlights an accidental death investigation involving an individual huffing with the use of a 30-gallon plastic bag. Both cases involved individuals who had a history of nitrous oxide abuse, drug abuse, and mental illness.

Conclusion/Discussion: The concentration range for postmortem cases (2.54–273.5 mcg/mL) was higher than that of DUID cases (2.29–8.01 mcg/mL), with some overlap. Due to the volatile nature of nitrous oxide, lack of information on the dose, and the varying length of time between collection and analysis, concentrations should be interpreted with caution. However, it should be noted that unless the patient had a recent medical procedure employing nitrous oxide the detection at any concentration is indicative of illicit use.

Keywords: nitrous oxide, laughing gas, huffing

S32 ERA Winner

Evaluating the Dragon in E-Cigarettes: An Analysis of the Aerosol from an E-Liquid Adulterated with Methadone

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Background/Introduction: Electronic cigarettes have been shown to produce ultra-fine aerosol particles facilitating the efficient absorption of drugs into the bloodstream. E-cigarettes are advertised to be a healthier alternative to cigarette smoking, but current generations are customizable, opening an avenue for abuse potential with Drugs Other Than Nicotine. Methadone is a schedule II opioid analgesic that is primarily used for opioid addiction maintenance, but is commonly abused because of its pharmacological similarity to morphine. Methadone is soluble in the propylene glycol, which is also a constituent in electronic cigarettes.

Objective: To determine the dose of methadone contained in the aerosol generated from an electronic cigarette at three concentrations and three voltages and to determine the particle size distribution of the methadone aerosol in comparison to drugs such as nicotine and methamphetamine.

Method: For dose capture analysis, e-liquid formulations were made to contain 10, 30, and 60 mg/mL methadone in propylene glycol and vegetable glycerin in a 50:50 (v:v) ratio. The e-liquids were "vaped" from a KangerTech AeroTank e-cigarette with a 1.8 Ω pre-assembled atomizer, and an eGo-V2 variable voltage battery for 4 sec at 2.3 L/min. The voltage of the e-cigarette battery varied from 3.9-4.7 V and each e-liquid concentration and voltage were replicated five times for a total of 45 samples. The aerosolized product was captured using a water trap and the water from the trap was analyzed for methadone using a liquid-liquid extraction. For particle size distribution, the 60 mg/mL methadone e-liquid was generated at 4.3 V onto a 10-stage Micro Orifice Uniform Deposit Impactor (MOUDI) Model 100 at a flow rate of 30 mL/min. Each stage of the MOUDI represented a different particle size range, from 0.05-18 µm. Glycol deposition was determined by gravimetric analysis, while methadone deposition was determined using GC-MS. An Agilent GC-MS 6890N/5973 Mass Selective Detector (MSD) instrument with an Agilent HP-5MS column (0.25 mm x 30 m x 250 µm). Each sample was analyzed in split mode of 20:1. The initial temperature was set at 225 °C with a temperature ramp of 15 °C/min until 285 °C/min for a total run time of 4 minutes. The MSD was run in SIM mode.

Results: The theoretical yield of methadone was determined using the difference in tank weight of the e-cigarette before and after aerosol generation. The calculated dose-per-puff ranged from 38-549 µg across the 3 concentrations of e-liquids, and, quantitation determined the methadone dose in the aerosol ranged from 4-417 µg. Percent recovery of the aerosolized methadone ranged from 8-79%. Statistical analysis of the difference between the doses of the aerosolized methadone with increasing voltage showed a statistically significant difference between 3.9 and 4.7 V and 4.3 and 4.7 V ($P < 0.001$). The glycol gravimetric results and the quantitation of methadone on each stage of the MOUDI resulted in an 88-94% and 81-86% aerosol deposition onto the 0.172-0.31 µm stage, respectively. The Mean Mass Diameter of the aerosol containing methadone was 0.64 ± 0.16 µm.

Conclusion/Discussion: The dose of methadone in the aerosol and the size of its aerosol droplets was characterized in this study. The low dose of methadone in the aerosol and the wide variability in recovery could be contributed to the temperature output of the coils being lower than the boiling point of the drug. Poor aerosolization has been seen with other high boiling point compounds such as heroin. However, at a mean mass diameter less than 1 µm in size, the methadone can readily deposit into the lung tissue and be absorbed into the bloodstream. This study highlights the potential abuse that could be seen with opioids and e-cigarettes.

Keywords: E-cigarettes, methadone, particle size

Funding: This project was supported by Awards No. 2014-R2- CX-K010 and 2016-DN-BX-0150, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice and National Institute of Health (NIH) Center for Drug Abuse grant P30DA033934. The opinions, findings, and conclusions or recommendations expressed in this publication/program/exhibition are those of the author(s) and do not necessarily reflect those of the Department of Justice.

S33

Tracking Trends in Novel Psychoactive Substances in 2017 and 2018: Novel Illicit Opioids, Synthetic Cannabinoids, Benzodiazepines, and Cathinones

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Background/Introduction: Novel Psychoactive Substances (NPS) are substances that have emerged onto the recreational drug scene that may have either been chemically modified from existing drugs of abuse or repurposed from pharmaceutical or academic research for abuse often with the intent to skirt legal controls. NPS are very diverse in terms of drug class, effects and chemistry, and the majority of those identified to date mimic the effects of the six main groups of substances controlled under international drug conventions, including opioids, synthetic cannabinoid receptor agonists, stimulants, opioids, dissociatives, classic hallucinogens, and sedatives/hypnotics. Although more substances are reported each year, many NPS are transient in nature, and others remain available on illicit drug markets for a few years until they become regulated, after which their popularity tends to drop off as new substances emerge. Diverse temporal and geographic trends of NPS emergence are observed, both in terms of the substances themselves the regions where they are identified. Toxicological data identifying NPS use in postmortem and human performance casework provides intelligence for law enforcement and public health agencies. Toxicological data for multiple synthetic substances are monitored over time and shifts in popularity noted, typically in response to some type of regulatory control.

Objective: This presentation describes the timeframes in which new substances have emerged in the United States in 2017 and 2018.

PLATFORM

Method: Analytical and client data from January 2017 through March of 2018 from a large reference laboratory was reviewed to document the presence of NPS classes including opioids, benzodiazepines, synthetic cannabinoids, and stimulants in blood specimens obtained during the course of death investigation, human performance, and suspected overdose casework.

Results: The most frequently encountered NPS in each category for 2017/Q1 2018 were as follows. A total of 300 cases were positive for the following designer benzodiazepines: etizolam (172, 57.3%), delorazepam (58, 19.3%), flubromazolam (55, 16.4%), diclazepam (29, 9.6%), and flubromazepam (25, 8.3%). A total of 127 cases were positive for the following cathinone derivatives: N-ethylpentylone (89, 70.0%), alpha-PVP (31, 24.4%), dibutylone (8, 6.2%), and butylone (4, 3.14%). A total of 453 cases tested positive for the following synthetic cannabinoids: 5F-ADB (212, 46.7%), FUB-AMB (109, 24%), ADB-FUBINACA (93, 20.5%), and AB-FUBINACA (25, 5.5%). A total of 5992 cases tested positive for designer opioids and fentanyl analogs:: acetylfentanyl (2233, 37.2%), furanylfentanyl (1143, 19.0%), para-fluoroisobutyrylfentanyl/FIBF (994, 16.5%), carfentanil (716, 11.9%), U-47700 (655, 10.9%), cyclopropylfentanyl (628, 10.4%), methoxyacetylfentanyl (359, 5.9%), acrylfentanyl (330, 5.5%), butyry/isobutyrylfentanyl (170, 2.8%), and 3-methylfentanyl (113, 1.8%). In addition 23 cases have been confirmed positive for the designer hallucinogen 3-methoxyphencyclidine.

During the first half of 2017, furanylfentanyl and U-47700, respectively, were the frequently reported novel illicit opioids. However, following scheduling of these compounds in China in May and July of that year, the positivity rates dropped off while para-fluoroisobutyrylfentanyl/FIBF and cyclopropylfentanyl increased in popularity. Cyclopropylfentanyl and methoxyacetylfentanyl are the most commonly reported novel opioids as of March 2018, lagging behind only acetylfentanyl, which has shown a resurgence in popularity from its appearance in the market in 2013.

Conclusion/Discussion: This data provides insight into patterns of use for the multiple categories of NPS substances. By correlating positivity rates with other data, such as scheduling dates and the jurisdiction where the cases came from, both public health and public safety agencies can stay abreast with changing patterns in the recreational use of these substances. Further investigation will be conducted through 2018 to expand the evaluated timeline and allow for new compounds to be investigated as they arise.

Keywords: NPS, Opioids, Cannabinoids

S34

Dangerous Concoctions? The Alkaloids, Bacteria, and Pesticides in Traditional Chinese Herbal Medicines

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Background/Introduction: Traditional Chinese Medicine (TCM) is rooted in ancient Chinese culture and dates back more than 2,500 years. An estimated 50% of the US population consumes herbal medicines. Often believed to be safer with less adverse effects, some herbal products require a Chinese physician and/or pharmacist's prescription while others can be purchased over-the-counter. The ingredients of TCM are often unknown or are inconsistent with labeling, and are, in some cases, less expensive than traditional pharmaceutics. These herbal products are considered dietary supplements under the Dietary Supplement Health and Education Act of 1994, meaning they are considered safe to consume until an epidemic of toxicological emergencies and/or deaths occurs, deeming them unsafe. Reports of TCM products have claimed toxic contaminants including heavy metals, pesticides, and in some cases, pathogenic bacteria.

Objective: The purpose of this study was to preliminarily identify the pharmacologically active alkaloids in TCMs, any microorganisms on the plant material, and any organochlorine pesticides still present.

Method: Eleven herbs classified as sedative hypnotics or anticonvulsants were chosen from *The Pharmacology of Chinese Herbs*. These include: *Ziziphus spinosa, Poria cocos, Astragalas complanatus, Pheretima asperigillam, Uncaria rhyschophylla, Gastrodia elata, Bombyx mori, Corydalis turtschaninovii, Corydalis incisa, Lindera strychnifolia,* and *Commiphora myrrha.* They were purchased from Tong Ren Tang (Beijing, China). Each herb was wrapped in brown paper with the manufacturer's information as well as brewing instructions printed in Chinese.

Alkaloid characterization of dried TCM and TCM extracts was performed using a Direct Analysis in Real Time Mass Spectrometer (DART-MS). Analysis of microbial/fungal lipids from the herbs was conducted using Fatty Acid Methyl Ester (FAME) Profiling with Gas Chromatography-Flame Ionization Detector (GC-FID). Pesticide extraction was completed with a modified QuECHERS method and substances presumptively identified on a Gas Chromatography-Mass Spectrometer (GC-MS).

Results: At least one expected alkaloid was presumptively identified in nine of the eleven TCMs, including nuciferine, vanillin, corydaline, tumulosic acid, z-guggusterol, cheilanthefoline, hirsutine, corynantheine, hypoxanthine, guanidine, succinic acid, and arachidonic acid. Common strains of the genus bacteria, *Bacillus*, was presumptively identified in five out of eleven herb cultures. A gram-positive, aerobic bacteria related to *Bacilli, Paenibacillus thiaminolyticus, was also presumptively determined.* Fungal growth was also determined to be present by the identification of polyunsaturated fatty acids common to eukaryotes. Organochlorine pesticides were presumptively identified on two herbal products, *Ziziphus spinosa,* or "Suan Zao Ren" and *Pheretima asperigillam*, or "Di Long".

Conclusion/Discussion: The methods described were effective in helping to understand the extent of contamination present in Traditional Chinese Medicine. The combination of active alkaloids like the sedative nuciferine, with microorganisms, such as *Paenibacillus thiaminolyticus* reported to cause bacteremic infections in humans, can lead to unexpected health emergencies. Potentially exacerbating a health emergency could be the ingestion of pesticides demonstrated to also exist on the herbal products. The combination of contaminants, particularly for an immuno-compromised person, could be deadly. Therefore, the identification of the active alkaloids, microorganisms, and pesticides in TCM can be useful in the reconstruction of toxicological episodes or deaths that occur after the use of these products.

Keywords: Chinese herbs, microorganisms, pesticides

PLATFORM

User Reports Versus Analytical Confirmation Of Novel Opioid Ingestion Following Hospitalization

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Background/Introduction: Novel opioids, now including a long list of fentanyl analogues, have caused widespread adverse events, including tens of thousands of deaths, in the United States and around the world. Contrarily, there exists a large clinical population that is under studied upon presentation at emergency departments (ED) following suspected opioid overdoses. In both instances, drug users are frequently unaware of the substances they are ingesting and, when able, often give incorrect or misleading information to emergency medical providers after suffering adverse effects. In addition, due to limited resources for toxicology testing and symptomatic treatment of overdose victims, there is usually no toxicological or chemical confirmation of the substance(s) ingested.

Objective: We describe a collaboration between ED physicians, medical toxicologists, and forensic toxicologists to correlate clinical findings, patient histories, and self-reports of drug use with toxicological confirmations for novel opioids following suspected opioid overdose, naloxone administration, and ED admission.

Method: Patients (n=3) admitted to the ED of a large urban Massachusetts hospital following suspected opioid overdose were recruited for an IRB-approved study to provide information regarding their drug use, as well as to provide blood or urine specimens. Biological specimens were screened via liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF) using a Sciex TripleTOF® 5600+ for the presence of more than 450 therapeutic, abused, and emerging drugs. Qualitative confirmation was performed using a separate, distinct LC-QTOF method on a Water Xevo® G2-S. Quantitative confirmation for select novel opioids was performed via liquid chromatography tandem mass spectrometry (LC-MS/MS) using an Agilent 6430.

Results: Patient 1 was a 23-year-old male who overdosed following insufflation of "Synthetic Heroin China White #4" purchased through the Dark Web and shipped via the US Postal Service. He insufflated roughly 20 "tiny bumps" (micro-doses) of the substance prior to experiencing overdose symptoms. Emergency medical services (EMS) were called and administered naloxone. Urine was collected at the hospital and independent laboratory testing (LC-QTOF) showed the presence of furanyl fentanyl, 4-ANPP, naloxone, and other drugs of abuse.

Patient 2 was a 29-year-old female in an outpatient facility for previous issues related to drug use. She reported intravenous heroin use but noted that the substance differed in color from previous experiences. Following experienced symptoms of overdose, EMS were called and administered naloxone to the patient. Urine was collected at the hospital and independent laboratory testing (LC-QTOF) showed the presence of fentanyl, norfentanyl, 6-MAM, morphine, codeine, and naloxone.

Patient 3 was a 17-year-old male who received crushed "Xanax" tablets from a friend. He reported insufflating the powder in his bedroom prior to getting in the car with his mother to go to school. En route, the patient began to show symptoms of a drug overdose and was ultimately administered naloxone by the school nurse upon arrival. Blood and urine were collected at the hospital and independent laboratory testing (LC-QTOF and LC-MS/MS) showed the presence of U-47700 (282.4 ng/mL in blood) and its metabolites, as well as naloxone.

Conclusion/Discussion: These cases, including histories and self-reports, along with corresponding toxicology confirmation results demonstrate that user accounts of naloxone-reversed overdoses rarely give accurate information about the substances ingested. However, since this population is not routinely tested, details are not typically available regarding the nature of drug(s) ingested and drug combinations used in survived overdoses. While the results show self-report cannot be relied upon solely, self-reports do provide interesting circumstantial information for toxicologists. Additional testing of clinically poisoned patients who appear to be suffering opioid overdoses will continue to provide valuable insight into patterns of drug use, which can ultimately complement information from fatal drug overdoses.

Keywords: Novel opioids, overdose, naloxone

S36

Getting Fat Out of Fat: The Extraction of Cannabinoids from Chocolate

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Background/Introduction: In recent years, the number of states that have legalized marijuana has increased. Currently 30 states and the District of Columbia have legalized the use of marijuana for medicinal purposes. Recreational use of marijuana has been legalized in 8 states and the District of Columbia. While marijuana is federally scheduled by the US Drug Enforcement Administration (DEA) as a Schedule 1 substance, the only retail or medicinal formulation of marijuana products the US Food and Drug Administration (FDA) regulates is Marinol®. The FDA does not regulate the production of marijuana edibles or "medibles", such as candies and baked goods. Medibles primarily contain delta 9-tetrahydrocannabinol (THC), the psychoactive component of marijuana, and cannabidiol (CBD), another cannabinoid which has reputed medicinal properties. Previous work has been performed to determine appropriate extraction techniques for high fiber and high sugar materials. This study will focus on appropriate extraction techniques for high fat (dark chocolate) cannabinoid products. Previous work did not include other cannabinoids, such as cannabigerol (CBG), cannabichromene (CBC), cannabicyclol (CBL), cannabinol (CBN), tetrahydrocannabivarin (THCV), tetrahydrocannabinolic acid (THCA-A), and cannabidolic acid (CBDA).

Objective: To evaluate sample preparation techniques for the extraction of cannabinoids from chocolate matrix material using a previously validated ultra-high pressure liquid chromatography tandem mass spectrometry method (UPLC-MS/MS).

Methods: Based on previous work with high sugar materials, a dissolution study was performed using 25 mg chocolate and 2 mL of solvent (i.e. acetonitrile, ethyl acetate, 9:1 hexane:ethyl acetate, methanol, methylene chloride, or water). The matrix was fortified to 8 mcg/g with the previously mentioned cannabinoids including isotopically labeled standards. A previously presented solid phase extraction (SPE) method for extraction of cannabinoids from high sugar materials was evaluated. In this method, 25 mg of chocolate was mixed with 0.5 mL water and heated at 58 °C for 5 minutes. Then 0.5 mL acetonitrile was added and the sample was passed through a Clean Screen FASt® column (United Chemical Technologies (UCT), Bristol, PA). The SPE column was also evaluated using 25 mg of chocolate and 1 mL of solvent (ethyl acetate, hexane, and 9:1 hexane:ethyl acetate). The eluate was evaporated to dryness and reconstituted with mobile phase. The necessity of additional elution solvent was also evaluated. The effect of pH was evaluated using the addition of ammonium hydroxide or hydrochloric acid to the organic solvent. Finally, a comparison of two different UCT columns (Clean Screen FASt® or FASt® THC) was performed. Chromatographic separation was performed using a Zorbax XDB-C18 4.6x75mm column and a 0.1 mM ammonium formate water:methanol mobile phase The mobile phase was 0.1 mM ammonium formate in water (A) and 0.1 mM ammonium formate in methanol (B) isocratic at 10:90 for 6 min followed by a gradient to 0:100 over 1 min. The column flow rate was 0.5 mL/min. The acquisition mode was multiple reaction monitoring, and positive mode electrospray ionization.

Results: Complete dissolution of chocolate was observed using methylene chloride, however degradation of the cannabinoids occurred. Using the high sugar method, there was insufficient clean-up using the FASt® columns, resulting in a tan colored eluate. Based on peak height, ethyl acetate on the FASt® column was determined the most effective for the relative recovery of the cannabinoids, however THCA-A and CBDA were not recovered. The addition of 1 mL of organic solvent added after the initial addition of the sample solution to the FASt® column increased recovery. Buffering the solution did not increase recovery of THCA-A or CBDA. The FASt® THC column did allow for the recovery of all cannabinoids and an acidic pH increased recovery of all cannabinoids including the THCA-A and CBDA.

Conclusions/Discussions: Ethyl acetate was the optimum solvent for dissolution of the chocolate matrix. The UCT FASt® THC columns with an acidic pH yielded the highest recovery of all cannabinoids evaluated.

Acknowledgements: This project was supported by the National Institute of Justice, Research and Development in Forensic Science for Criminal Justice Purposes, 2017-R2-CX-0029.

Keywords: Cannabinoids, Medibles, Sample Preparation

S37

Simultaneous LC/MS/MS Monitoring of Thermal Degradation Products and Parent Synthetic Cannabinoids in Postmortem Blood Samples

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Background/Introduction: Synthetic cannabinoids are potent agonists at the cannabinoids receptors, which are abused as a substitute to Δ^9-tetrahydrocannabinol (THC); however, acute toxic effects including tachycardia, seizures, possible suicidal tendencies, and psychotic episodes occur. Given that the primary mode of ingestion is inhalation through smoking, understanding the pyrolysis of synthetic cannabinoids is necessary. A previous study has been conducted and numerous thermal degradation products were identified. These pyrolytics may have activity and be toxic to users and innocent bystanders. To explore this possibility, analysis of post mortem samples to establish the ingestion of thermal degradants along with the parent compounds was carried out.

Objective: To demonstrate the presence of thermal degradation products within post mortem samples, which were previously analyzed and shown to be positive for synthetic cannabinoids. This is the first simultaneous monitoring of both degradants and parent cannabinoids to date. The detected pyrolytics were semi-quantified, and establish that they cannot be dismissed when evaluating possible toxicological effects.

Methods: An extraction protocol and LC/MS/MS method was adapted from a previous report and validated for the analysis of 23 post mortem blood samples. These samples had been previously analyzed and 19 contained a single synthetic cannabinoid, while the remaining 4 were mixtures. Method validation, including compound optimization, extraction efficiency, matrix effect, linearity, precision and accuracy studies were conducted on the present cannabinoids: 5F-ADB, 5F-AMB, 5F-PB-22, FUB-AMB, FUB-AKB-48, AB-CHMINACA, AB-FUBINACA, AB-PINACA, ADB-CHMINACA and ADB-FUBINACA. The extraction protocol utilized a buffer pre-treatment followed by a hexane-ethyl acetate liquid-liquid extraction. Linearity studies were then performed using a concentration range of 0.01-1000 ng/mL, and evaluated over a 10 day period. The precision and accuracy studies were assessed at a low, mid and high concentration, and the extraction efficiency and matric effects were tested at a low and high concentration of the linearity range. The 23 obtained post mortem samples were analyzed and the pyrolytic compounds present were identified. All of the analyses were carried with a Shimadzu LC-20 UPLC system coupled with a SCIEX 3200 Q-Trap® mass spectrometer.

Results: All of the validation parameters were met based on SWGTOX guidelines. The blood samples were analyzed in triplicate, and thermal degradants were observed in each sample except 1, which had a small sample volume. A number of pyrolytics were consistently observed, and their presence was semi-quantitated. The determined presence of such products in a toxicological specimen is pertinent in establishing that such products cannot be dismissed in toxicology assessments. Data analysis of single drug samples in comparison to mixtures, allowed for the connection between parent synthetic cannabinoid and thermal degradation product.

Conclusion/Discussion: The presented study demonstrates the detection of thermal degradation products in post mortem blood samples via LC/MS/MS. The observed pyrolytic products demonstrate the requirement of additional research endeavors to fully understand their impact on acute toxicity, pharmacodynamics activity and possible metabolic profile. The pyrolytics additionally more closely resemble serotonin, structurally, which may strengthen hypotheses of cannabinoid toxicity having symptom overlap with serotonin syndrome.

Key Words: Synthetic Cannabinoids, Pyrolysis, Post Mortem Analysis

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

S38

Mitragynine (Kratom)- and Cyclopropyl Fentanyl-Related Deaths in Oregon

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Background/Introduction: In August of 2016, the DEA announced its intention to classify Mitragynine (sold as Kratom) as a Schedule I drug. They noted that between 2000 and 2005 only two exposures to mitragynine were reported; between 2010 and 2015, 660 calls were made to US poison control centers. The DEA was aware of 15 mitragynine-related deaths between 2014 and 2016. Congress interceded with a plea to delay scheduling it until more data was collected. They cited scientific research indicating mitragynine can be used as a non-addictive analgesic and contended that the licensing to continue research on a Schedule I drug would be overly burdensome. The DEA withdrew the plans to schedule Mitragynine at this time.

In November of 2017, the DEA announced its intent to schedule Cyclopropyl fentanyl as a Schedule I controlled substance. This temporary scheduling order went into effect on January 4, 2018. The DEA cited 115 confirmed fatalities associated with cyclopropyl fentanyl, all since 2017.

Objective: To describe the relevance of two drugs to postmortem cases in Oregon and demonstrate the benefit of a sensitive technique for screening and confirmation.

Method: Our laboratory has recently developed a screening method using LC-QTOF (Shimadzu Nexera LC-20ADXR system coupled with a Sciex 5600+ QTOF and associated software; Column: Phenomenex Kinetex 2.6 µm Biphenyl column (100 Å, 2.1 x 50 mm); Guard Column: Phenomenex SecurityGuard™ Ultra cartridge (UHPLC Biphenyl, for 2.1mm ID columns); Buffer A: 0.05% Formic acid in 5 mM ammonium formate, Buffer B: 0.05% Formic acid in 50:50 acetonitrile/methanol; SWATH acquisition method).

We have also developed a quantitative method for quantitation of both cyclopropyl fentanyl and mitragynine using LC/MS/MS (Agilent 1290 UHPLC coupled with ABSciex 3200 QTrap MS/MS and associated software; Column: Agilent Poroshell EC-C18, 2.6 µm, 2.1 x 50 mm; Monitored ions for cyclopropyl fentanyl: 349.2, 188.1, 105.0; Monitored ions for mitragynine: 399.2, 174.1, 226.3; Cyclopropyl fentanyl-D5 and mitragynine-D3 internal standards; Buffer A: 0.2% Formic acid in 2 mM ammonium formate, Buffer B: 0.2% Formic acid in 2 mM ammonium formate in acetonitrile).

Results: In Oregon, between October 24, 2016 and December 11, 2017, mitragynine was confirmed in eighteen deaths; in some of these deaths no other contributing drug was detected except a low blood alcohol level or indication of marijuana use. All but three of the victims were male and ranged in age from 27 to 48. The deaths occurred in ten different counties throughout Oregon. The blood concentrations ranged from 5.6 ng/mL to 29000 ng/mL.

In Oregon, between May 2, 2017 and January 12, 2018, cyclopropyl fentanyl was confirmed in thirty four deaths. All but seven of the victims were male and ranged in age from 2 months to 61 years. The deaths occurred in nine different counties throughout Oregon. The blood concentrations ranged from < 1.0 ng/mL to 26 ng/mL.

The antemortem toxicology group has also encountered both mitragynine and cyclopropyl fentanyl in DUII cases.

Conclusion/Discussion: Mitragynine and cyclopropyl fentanyl are difficult drugs to detect in blood using typical analytical instrumentation, so it is possible their contribution to drug-related deaths is underestimated. With the introduction of the LC-QTOF, we have a much more sensitive screening technique and are able to readily detect both of these drugs. Our quantitative method on the LCMSMS has a lower limit of quantitation of 1 ng/mL for cyclopropyl fentanyl and 10 ng/mL for mitragynine.

Keywords: Cyclopropyl fentanyl, mitragynine

5-Fluoro-ADB related fatality and intoxication in Singapore

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Background/Introduction: 5-fluoro-ADB (5-fluoro-MDMB-PINACA), an indazole-type synthetic cannabinoid, has been reported to have potency ~290 times that of THC [1]. Fatalities and intoxications related to 5-fluoro-ADB have been reported in Japan, Europe and the United States. In Singapore, 5-fluoro-ADB and/or its ester hydrolysis metabolite (M1) were detected in 23 (2 post-mortem and 21 ante-mortem) cases from October 2017 to March 2018.

Objective: To present cases in Singapore in which 5-fluoro-ADB and/or its ester hydrolysis metabolite were screened and confirmed in routine toxicology.

Methods: Ante-mortem cases with requests for general drug screening in urine are routinely subjected to both basic and acidic liquid-liquid extractions (LLE) for GC/MS and ion trap LC-MSn analysis. Both GC/MS (on Agilent system) and LC-MSn (on Bruker Toxtyper system) use spectral libraries containing ~850 (GC/MS) and ~1200 (LC/MSn) compounds with retention times, including some novel psychoactive substances. In most cases, 5-fluoro-ADB and/or M1 were detected by LC-MSn. For the 2 post-mortem cases where urine samples were not submitted, 5-fluoro-ADB was detected by GC/MS in the peripheral blood, stomach contents and lung tissue.

The presence of 5-fluoro-ADB and its metabolite M1 in these cases was further confirmed by an LC–high resolution orbitrap MS (Thermo Q-Exactive). Urine samples were enzymatically hydrolysed, diluted, filtered and injected into Q-Exactive. Quantitation of 5-fluoro-ADB and M1 in post-mortem peripheral blood was performed by LC-MS/MS using 3 MRM transitions for each compound. Extraction for 5-fluoro-ADB and M1 was done by LLE under basic condition and acidic conditions, respectively.

Results: The profile of 5-fluoro-ADB abusers in these 23 cases were mostly male, with ages ranging from 20 to 63 years old. Nitrazepam which is commonly abused amongst local abusers, was also detected in 48% (11) of the cases. M1 was detected in all 21 ante-mortem urine samples, with the parent compound detectable only in one urine sample. The results of the toxicology analysis of the two post-mortem cases were as shown in Table 1 below.

Table 1: Toxicology results of the post-mortem cases

Case description	Drugs detected
Case 1 A 49 yo female found dead in a room. Past history of inhaling toluene and suffering from schizophrenia.	Peripheral blood: 5-fluoro-ADB (<0.1 ng/ml), M1 (24 ng/ml), nitrazepam (60 ng/ml), zuclopenthixol (180 ng/ml) Stomach contents: 5-fluoro-ADB, nitrazepam, zuclopenthixol Lung tissue: M1
Case 2 A 55 yo male found by passer-by lying on a grass patch. Had previous history of unsound mind.	Peripheral blood: 5-fluoro-ADB (0.26 ng/ml), M1 (74 ng/ml), nitrazepam (210 ng/ml)

Conclusion/Discussions: The levels of 5-fluoro-ADB in the post-mortem blood specimens were found to be much lower than M1. This is consistent with the findings by Kusano et al. [1,2] where they attributed to the low blood 5-fluoro-ADB concentrations possibly to extensive enzymatic metabolism by carboxylesterases. Our cases demonstrated that 5-fluoro-ADB ester hydrolysis metabolite should be a target analyte for the detection of 5-fluoro-ADB use in both blood and urine specimens.

Keywords: 5-Fluoro-ADB, 5-Fluoro-ADB ester hydrolysis metabolite, LC-MSn

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S40

Interpretation of Fluoroisobutyryl Fentanyl in Forensic Casework

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Background/Introduction: Fluoroisobutyryl fentanyl (FIBF) is a Schedule I narcotic, and is classified with synthetic opioids, a widely abused group of drugs that are attributed to a significant rise in overdose drug deaths in North America. FIBF was first identified at the NYC OCME in May of 2017. The in-house LC/MS/MS method in place at that time was validated for the quantitation of a number of fentanyl analogs, including the FIBF isomer, fluorobutyryl fentanyl (FBF) but not FIBF. Suddenly, no further cases of FBF were identified and it was FIBF that increased in prevalence and therefore was added to the method for qualitative analysis. By the end of 2017, FIBF was the fifth most common non-methadone synthetic opioid detected in cases submitted to the OCME behind fentanyl, norfentanyl, 4-ANPP, and acetylfentanyl (in order of prevalence).

Objective: To validate a quantitative method for FIBF in blood and to re-test all cases from 2017 where FIBF was identified at the OCME in order to determine the blood concentrations associated with different forensic case types (ante mortem versus postmortem). Further, to assess the case demographics, understand what drugs are commonly associated with the presence of FIBF, and how this relates to the cause of death in postmortem cases.

Method: A quantitative method for FIBF determination in blood was developed and validated on an Agilent 6460 Triple Quad LC/MS. The method includes seven calibrators spanning the range of 0.1 – 100 ng/mL with a limit of detection at 0.05 ng/mL and quality controls tested at 0.5, 8 and 80 ng/mL. Acceptable limitations for all validation parameters were met according to SWGTOX guidelines. After validation of the method was complete, all 131 cases that tested positive by LC/MS/MS from May - December 2017 were chosen for re-analysis. Additionally, demographic and case history information was compiled for all cases where available.

Results: A total of 126 FIBF-positive cases had sufficient specimen volume available for re-analyzing and included DFSA (N=1), DWI (N=5), and post-mortem (N=120) cases. FIBF cases most commonly were male (82.5%), Caucasian (52.4%) and between the ages of 35 - 44 years (30.9%). FIBF was reported as contributing to the cause of death in 90% of the postmortem cases, and in only two cases it was the sole intoxicant responsible for death. The manner of death was most commonly accidental (94.9%) but there were two cases each attributed to homicides, suicides and natural deaths. As is frequently reported for other illicit drugs, FIBF was identified in combination with other drugs in all but one case in this study. The most prevalent drugs found included other opioids (95.2%), benzodiazepines (38.9%), cocaine (35.7%), cannabinoids (28.5%), and ethanol (27%). The blood concentration of FIBF measured ranged from < 0.1 – 243 ng/mL, with 44% of the concentrations < 1 ng/mL, 48% in the range of 1 – 50 ng/mL and 8% of cases with concentrations above 50 ng/mL.

Conclusion/Discussion: The concentrations of FIBF in blood varied widely across case types, and showed no clear distinction when considering the cause or manner of death, or for postmortem versus ante mortem cases. The two cases where acute intoxication with FIBF was reported demonstrates this; one case had a femoral blood concentration of 2.1 ng/mL while the second was 44 ng/mL. The synthetic opioid epidemic has presented forensic toxicology laboratories with a unique challenge when validating new methods to keep up with the ever changing emergence and subsequent disappearance of different "fentalogs". The development of a quantitative method for FIBF has been beneficial at the OCME due to the significant numbers of positive cases reported and will help inform future case interpretation.

Keywords: Fluoroisobutyryl fentanyl, postmortem, Synthetic Opioids

S41

Case Report: A Scuba Dive that Ended in Drowning by Chemical Inhalation

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Background/Introduction: The body of a 60-year-old female was recovered from the sea off River Bay, Saint Lucy, Barbados after she failed to surface following a scuba diving expedition. She was a well experienced scuba diver who made daily diving trips with her friends while retired in the Barbados islands. According to her computer's dive profile, she descended for about two minutes to 42ft where she presumably started to experience problems, ascended quickly to 27ft activating an ascent alarm, and then descended to 66ft three minutes into the dive; it is believed that she became unconscious during this descent. She was recovered 14 minutes later by members of her dive group. Analysis of postmortem blood and tissue revealed the presence of dichloromethane (DCM) a volatile organic compound used commercially as a paint remover and degreaser with similar toxicological effects as chloroform that can result in blackouts and unconsciousness. Follow up testing revealed the presence of DCM in the scuba tank. Testing methodology and the investigative process will be discussed.

Objective: The role of the investigative forensic toxicologist requires an understanding of the circumstances of death, the facts and history leading to the moment of death, and the environment in which death occurred. Piecing these elements together with a sound analytical approach can reveal many answers. The objective of this presentation will be to discuss this approach and how it was used to solve this deadly mystery.

PLATFORM

Method: Headspace gas chromatography (HS-GC-FID) was used initially to detect DCM in postmortem fluids and tissues during a routine blood volatiles screen. DCM in these fluids and tissues was identified in follow-up tests by headspace GC-MS to make a definitive identification. DCM was identified in the tank using both solid phase micro-extraction (SPME) and headspace GC-MS techniques. Quantitative measurements in the scuba air were made using chloroform as an internal standard and HS-GC-MS. Ideal Gas Law equations were used to estimate the amount of DCM in the scuba tank. Additional drug screens were performed on post-mortem blood and tissues by ELISA, GC-NPD and GC-MS as well as a screen for carbon monoxide by co-oximetry.

Results: DCM was detected in postmortem blood, brain, liver and ocular fluid. No quantitative measurements were made of the DCM in the blood and tissues due to sample quality. However, measurements of the DCM concentration in the scuba air was performed. The goal was to determine whether contamination of the air might have been intentional or accidental. The 80ft^3 scuba tank was received with 800psi of remaining air. The tank capacity when full was 3000psi. Consequently based on the measurements of the concentration of DCM in the remaining 800psi, the calculated volume of DCM in the tank when full was approximately 27mL. Additional drug and volatile screens on postmortem blood revealed ethanol (0.02%), codeine (0.006 mg/L), acetaminophen (17 mg/L) and traces of chlorpheniramine. Carbon monoxide, a metabolite of DCM, was not detected in the postmortem blood due to the quality of the samples.

Conclusion/Discussion: Understanding the cause of death in this case must also include an understanding of diving physiology and the change in breathing gas density at depth when scuba diving. At the surface, this volume of DCM would likely not be toxic but at 2 atmospheres (33ft) and 3 atmospheres (66ft) the amount (partial pressure) of DCM inhaled had increased significantly causing disorientation and unconsciousness. The likelihood of drowning becomes certain. The mystery of how the DCM got into the scuba tank may never be solved but the possibility of intentional contamination cannot be discarded.

Keywords: Scuba diving, diving physiology, drowning, dichloromethane, headspace gas chromatography-mass spectrometry

S42

Withdrawn

Development of Quantitative Formic Acid Analysis by GCMS for Urine, Serum/Plasma, and Blood

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Background/Introduction: Formic acid and its conjugate base formate are essential endogenous one-carbon metabolites in most living organisms participating in vital one-carbon pool of intermediary metabolism (1). A metabolite of methanol and formaldehyde, formic acid, is responsible for the toxic effect in methanol poisoning. Under normal conditions, methanol is metabolized to formaldehyde, then to formic acid, which breaks down to carbon dioxide (CO2) and water, and is partially excreted unchanged in urine. At toxic levels, formic acid inhibits the enzyme mitochondrial cytochrome oxidase, causing hypoxia at the cellular level and metabolic acidosis, among a variety of other metabolic disturbances.

Objective: To develop a new and sensitive method to quantitate formic acid in various matrices.

Method: Agilent 7890A Gas Chromatography coupled with Agilent 5975C Mass Spectrometry with a 7697A Headspace Auto-sampler was used to quantitate formic acid propyl ester in blood, urine, and serum/plasma for both antemortem (AM) and postmortem (PM) cases. The derivatizing reagent used is n-propanol combined with concentrated sulfuric acid (ratio 3.5:1). The internal standard (ISTD) chosen is an isotopically labeled internal standard, Formic-13C Acid. The column selected is Rtx-BAC Plus 1 from Restek with a calculated beta value of 44. The sample preparation includes the addition of sample, 0.1mg/mL ISTD and derivatizing reagent into a 20mL headspace vial. Urine and serum/plasma are run straight and blood has a default 1+1 upfront dilution to compensate the viscosity. A quantitative method has an analytical measurement range of 0.5 – 50 mcg/mL. Sensitivity was significantly improved by achieving the LOQ that is 10x lower than the current in-house urine method. The target ion for formic acid is *m/z* 47.1 with *m/z* 73.1 and 88 as qualifier ions. The target ion for the isotopic-labeled internal standard is *m/z* 48.0 with ions *m/z* 74 and 62 as qualifier ions.

Results: Anonymous samples were obtained from a pool of discard samples for which the requested testing was completed. The samples were not specifically submitted for suspected methanol poisoning, formaldehyde or formic acid exposure. Sample pools contained both male and female specimens from various age groups and were randomly selected based on criteria such as a source of specimen (antemortem vs. postmortem), type of matrix and the availability of specimen. The mean (±SD**)**, median and range of formic acid concentrations as well as previously published values are shown in Table 1.

	Number of cases analyzed	Mean (±SD) (mcg/mL)	Median (mcg/mL)	Range (mcg/mL)	Previously published values (mcg/mL)
Postmortem Blood	n = 30	4.5 (±2.0)	4.2	2.1 – 10.1	40 [2] (mean/median)
Postmortem Urine	n = 30	4.6 (±2.0)	4.2	1.4 – 38.2	60/40 [2] (mean/median)
Antemortem Blood	n = 29	4.3 (±0.85)	4.3	2.9 – 6.2	N/A
Antemortem Urine	n = 72	13.1 (±7.5)	11.1	2.1 – 10.1	2 – 30 [2] (range)
Antemortem Serum	n = 73	1.2 (±1.2)	0.92	0.5 – 9.3	44 [3] (upper range)

Table 1: Calculated formic acid concentrations for postmortem blood and urine, and antemortem blood, urine, and serum.

Conclusion/Discussion: The new and sensitive method for the analysis of formic acid was developed and used to quantitate formic acid in various matrices. The analysis suggested a lower concentration range for the normal population for antemortem urine and serum than previously reported values. Additionally, the mean and median concentrations for postmortem blood and urine were significantly lower than the previously reported values. The goal in the future is to analyze more samples for each subset using the newly developed method to establish a new reference range for normal population.

Keywords: Formic Acid, Formate propyl ester, Methanol poisoning

Reference:

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S44

Fully automated online extraction and analysis of opiates in urine by MPS-GC-MS

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Background/Introduction: The analysis of opiates in urine typically requires multiple sample preparation steps preceding any instrumental analysis. Currently, our laboratory uses an off-line automated solid phase extractor (SPE) for sample clean-up following an acid hydrolysis procedure. SPE is followed by solvent evaporation with subsequent derivatization and reconstitution to produce a sample extract suitable for gas chromatography-mass spectrometry (GC-MS) analysis. This process involves multiple steps performed by a trained personnel and is expensive, laborious, and time-consuming. The project described here employs automated sample processing utilizing Disposable Pipette Extraction (DPX) with Gerstel's MultiPurpose Sampler (MPS) coupled to an Agilent GC-MS system for the extraction of codeine and morphine from urine.

Objective: The objective of this study is to develop and validate a fully automated on-line analysis for the detection and identification of codeine and morphine in urine using a DPX-MPS-GC-MS analytical procedure.

Method: A dual head MPS system with 98-position 2ml vial trays, SPE module and the necessary solvent and waste reservoirs were used for the automated sample extraction, solvent evaporation, derivatization, reconstitution and sample injection. DPX tubes which contained loosely packed reversed phase salting (DPX-RP-S) sorbent were used for the extraction.

Prior to loading onto the MPS system, the urine samples (0.25 ml) with deuterated internal standard (0.05 ml) were acid hydrolysed by the addition of 0.25 ml concentrated HCl and incubation at 99°C for an hour. The pH of each hydrolysed sample was adjusted to approximately pH 9 using ammonia solution (~12% w/w) and 6M HCl. An aliquot of 0.4 ml of each urine sample was then transferred to a clean culture tube and loaded onto the MPS system. Using the MPS, 0.8 ml of acetonitrile was added to the culture tube from which 1 ml of the solution was aspirated into a DPX-RP-S tip, followed by 1.3 ml of air to agitate the sorbent mixture. The sample was then dispensed back to the culture tube and the procedure was repeated for better mixing. Subsequently, the upper organic layer was transferred to a clean vial, dried under a stream of nitrogen at 75°C and derivatized using BSTFA with 1% TMCS and ethyl acetate at 75°C for 10 minutes. Ten µl of the derivatized sample was introduced into the GC/MS using a Multi-Mode Injector (MMI) with solvent vent mode. Quantitation and identification were performed with the GC/MS operated in selected ion monitoring (SIM) mode, with 1 target ion and 2 qualifier ions for codeine-TMS and morphine-2TMS.

Method development included optimising the alignment settings of the MPS system (e.g. needle depth for drawing of sample or reagent) and the extraction efficiency.

Results: This new method is able to achieve approximately 30 minutes for the on-line sample preparation and 10 minutes for the GC/MS analysis. The system allows concurrent extraction of one sample and GC/MS analysis of a previous extracted sample, thereby shortening the total analysis time to 30 minutes per sample.

Evaluation of the calibration linearity demonstrated acceptable regression coefficients ($r^2 \geq 0.9940$) for codeine and morphine concentrations ranging from 0.5 to 8 µg/ml. The limit of detection (LOD) and limit of quantitation (LOQ) were determined to be 0.5 µg/ml for both codeine and morphine. The final method will be validated for quantitative analysis according to the SWGTOX Standard Practices for Method Validation in Forensic Toxicology, and will include accuracy, precision, carryover, interferences, robustness and recovery. The applicability of the validated method for routine use will also be evaluated with a parallel study against the existing SPE method using routine urine samples collected from opiates abusers.

Conclusion/Discussion: The DPX-MPS-GC-MS method was successfully configured and developed for the automated on-line analysis of codeine and morphine in urine.

Keywords: MultiPurpose Sampler (MPS), Disposable pipette extraction (DPX), Opiates

S45

Development and Validation of a Novel All-Inclusive LC-MS/MS Designer Drug Method

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Background/Introduction: Designer drugs including synthetic cannabinoids (K2) and synthetic cathinones (CAT) are an increasing problem due to the ease of access to these compounds. They present analytical challenges inasmuch as the compound structures are numerous and growing within each class. Typically, each class of designer compounds is analyzed separately due to differences in chemistry, desired cut-offs, or other reasons. Physicians treating "high risk" patients typically order tests for all "illicit" substances which can span several test classes. Despite that multiple classes of designer drugs are ordered together, there has not been a comprehensive confirmatory test developed to date. Presented here is a novel comprehensive designer drug LC-MS/MS method that combines synthetic cannabinoids and synthetic cathinones, etizolam, a designer benzodiazepine and mitragynine (kratom), a natural product analgesic. This method improves laboratory throughput by combining analysis into a single method with a cycle time of 4.2 minutes which affords resolution of crucial isomers, such as ethylone and butylone. Development of this method was also an opportunity to update the list of compounds within the method. Analytes with fewer than 5 positive specimens in a year of testing with previous separate methods were removed. New analytes were added based on reports from NMS Laboratories and the United States Drug Enforcement Administration testing and drug seizures.

Objective: To develop a rapid LC-MS/MS semi-quantitative confirmation method for multiple classes of novel psychoactive substances (NPS) for urine drug testing. This new method was used to improve laboratory throughput and provide an opportunity to update the list of compounds to be more relevant.

Method: Reference standards were purchased from Cerilliant (Round Rock, TX), Lipomed (Cambridge, MA), and Cayman Chemical (Ann Arbor, MI). All solvents were purchased from VWR (Radnor, PA). Normal drug-free urine was purchased from UTAK (Valencia, CA). A recombinant β-glucuronidase, IMCSzyme®, was purchased from IMCS (Irmo, SC). All samples were analyzed via LC-MS/MS using an Agilent 6460 MS/MS with Agilent 1290 Infinity LC Stack. A Phenomenex Kinetex® XB-C18, 1.7 µm, 50 x 2.1 mm analytical column was used for chromatographic separation with solvents A: 0.1% formic acid in 90:10 water: methanol and B: 0.1% formic acid in methanol. The approximate cycle time for this method was 4.2 minutes. Prior to analysis, 40 µL of a 10,000 U/mL β-glucuronidase solution in 0.02 M phosphate buffer pH 7.5 and 40 µL of a methanolic internal standard solution was added to 300 µL of sample. Samples were then incubated in an oven for 35 minutes at 60°C and extracted on an Automated Liquid Dispenser III (ALD, SPEWare, Baldwin Park, CA) using CEREX® PSAX 96-Well NBE (narrow bore extraction) plates (SPEWare, Baldwin Park, CA). Following elution from the extraction, samples were dried down and reconstituted in 150 µL of 75:25 water: methanol. Two transitions were monitored for each analyte and internal standard.

Results: Method development focused on the liquid chromatography and the solid-phase extraction parts of the method. The original K2 and CAT methods utilized C18 column chemistry and similar solvents, leading to the selections of the Phenomenex column and solvents listed above. While the method was longer than either separate method, the separation of isomers, particularly the ethylone and butylone pair, was crucial to the success and led to a cycle time of 4.2 minutes. This also showed that the method was devoid of any interferences tested among common prescription and illicit drugs prevalent in our pain management population. As the CAT standalone method was dilute-and-shoot, the K2 extraction method was tested for compatibility with the CAT and newly added compounds. The method was modified as minimally as possible knowing the need for the low limits of detection for the K2 compounds. However, the α-hydroxy etizolam was not recovered with the original method. This required reducing the methanol concentration in one of the washing solvents to aid in recovery of α-hydroxy etizolam. Additionally, the high organic content in the reconstitution solution created chromatographic issues for the early eluting CAT compounds and was reduced to help balance the peak shapes with the solubility of the K2 compounds. Following development the method was validated according to the College of American Pathologists requirements, including limits, linearity, precision & accuracy, hydrolysis efficiency, matrix effect combined with extraction recovery and process efficiency, interference, and patient evaluation as able. All compounds met criteria for a semi-quantitative method, however, due to the lower sensitivity of the Agilent 6460 system compared to the Agilent 6490 system, the lower limits and subsequently reporting cut-offs for the K2 compounds were raised to pass validation. After validation the method was implemented into production testing and the impact of the reporting cut-offs was assessed.

In the first four months of testing, the most prevalent positive analytes were the newer K2 added to the method (Table 1). The overall positivity for the method increased from about 2.5 positives/week in the previous testing (~15 months CAT, ~4.5 years K2) prior to implementation of the updated method to ~2 positives/week for CAT and ~12.8 positives/week for K2 with the change in the analyte list in four months.

Conclusion/Discussion: Overall, a time efficient LC-MS/MS method was developed and validated to test for combined NPS analysis. This improves laboratory throughput and assists with specimens that have limited sample to use for multiple tests. While the validated cut-offs for the synthetic cannabinoids are higher than other methods, it does not appear to have affected the ability to detect the compounds in urine based on the patient data collected thus far.

Keywords: Synthetic cannabinoids, Synthetic Cathinones, Novel Psychoactive Substances, LC-MS/MS

Stability of the Designer Benzodiazepines Diclazepam, Flubromazepam and Etizolam in Solvents

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Background/Introduction: Designer benzodiazepines are of increasing forensic importance due to their rising popularity in the drug culture with diclazepam, flubromazepam and etizolam being some of the more prevalent. Understanding the stability of these compounds in solvents is necessary to ensure the accuracy of their identification and quantitation, helping prevent the use of inaccurate working solutions or degraded processed samples.

Objective: The aim of this study was to determine the stability of flubromazepam, diclazepam and etizolam when stored in either methanol or acetonitrile at room (21°C), refrigerator (4°C), and freezer (-20°C) temperatures.

Method: The stability of diclazepam, flubromazepam and etizolam in acetonitrile and methanol at room (21°C), refrigerator (4°C), and freezer (-20°C) temperatures was determined by periodically testing sample aliquots of known concentration over 30 days. Samples (n=3 per time point, per drug) were stored under the appropriate conditions, as individual 100 mL aliquots, evaporated to dryness on the appropriate day after the addition of diazepam-D_5 as internal standard and reconstituted in ethyl acetate for analysis by GC/MS. Analysis was carried out on a Perkin Elmer Clarus® 680 gas chromatography system with a Perkin Elmer Clarus® SQ 8 T Mass Spectrometer using a Perkin Elmer Elite-5 column (30m x 0.25 mm x 0.1 μm). Relative response area ratios of drug to internal standard were used in place of calculating concentrations, as the working solutions used in the calibration curves were stored in conditions involved in the stability study. As such any degradation experienced by the stability samples would occur at the same rate in the working solution, which would result in the concentrations calculated from the calibration curves staying constant over the course of the study period, incorrectly leading to the interpretation that the compounds were stable when that may not be the case.

Results: One-way analysis of variance (ANOVA) statistics with a significance value of 0.05 were applied to the stability study results to determine if the compounds were stable over the 30-day period. Results were also examined to see if they dropped below 20% of the original value during the course of the study, as this is the cutoff amount of variation marking the compound as unstable. Results indicated that diclazepam was stable at all temperatures in both solvents. Flubromazepam and etizolam, were both found to be unstable at all temperatures in both solvents. Between both solvents and all three storage conditions, flubromazepam lost bewteen 48.5-59.8% of its original value between days 0 and 30 and etizolam lost between 66.6-76.5% its original value between days 0 and 30, with the exception of flubromazepam at room temperature, both compounds dropped below 20% of the original response ratio between 0 and 3 days followed by a slower rate of decrease.. There was no significant difference in results between the two solvents or the three temperatures.

Conclusion/Discussion: The significant instability in flubromazepam and etizolam, could have major implications for laboratories reporting sample concentrations for these drugs. As the general practice is for working solutions to expire a year after preparation unless otherwise noted, it is possible that calibration curves for these compounds will over-estimate sample concentrations since the true working solution concentration is likely below its assumed concentration. Further work is planned to determine the impact of biological matrices upon the stability of these compounds as well as to determine the effects of light and air on the stability of these drugs as the certified standards are noted to be stable.

Keywords: Designer Benzodiazepines, Stability, Gas Chromatography-Mass Spectroscopy

PLATFORM

Comparison of blood and urine screening for drugs in DUI cases

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Background/Introduction: In Sweden, to be prosecuted for driving under the influence of drugs (DUI) you need to present with a scheduled drug in your blood. As part of the investigation a screening of drugs is performed using immune assays in either blood or urine. In an effort to conform the routines and recommend the best screening matrix we performed a study comparing the specificity and sensitivity of two screening approaches.

Objective: The objective of this study was to compare the two matrices blood and urine for immunochemical screening of carboxy-tetrahydrocannabinol (THC-COOH), d-amphetamine, opiates (morphine), cocaine (benzoylecgonine), benzodiazepines (oxazepam), methadone, buprenorphine, tramadol and 3,4-methylenedioxymethamphetamine (MDMA) in DUI cases

Method: Consecutive samples from DUI cases were used for the comparison. Depending on drug group, 868 to 1055 matched samples were analyzed using the routine urine and blood screening panels. The urine screening was based on the EMIT technology for amphetamine and morphine and the CEDIA technology for the remaining substances and analyzed on an ADVIA1800 instrument. The blood screening was based on the TECAN EVOlyzer platform and ELISA reagents from Immunalysis. The cutoffs in urine (first number) and blood (second number) were the following for THC-COOH 25 ng/ml and 10 ng/ml, amphetamine 300 ng/ml and 20 ng/ml, morphine 50 ng/ml and 10 ng/ml, benzoylecgonine 150 ng/ml and 20 ng/ml, oxazepam 200 ng/ml and 5 ng/ml, methadone 300 ng/ml and 50 ng/ml, buprenorphine 10 ng/ml and 1 ng/ml, tramadol 200 ng/ml and 50 ng/ml and MDMA 300 ng/ml and 10 ng/ml, respectively. All positive screening results were confirmed using routine mass spectrometry methods.

Results: In the blood screening 513 were positive for THC, 226 for amphetamine, 50 for morphine, 118 for benzoylecgonine, 240 for benzodiazepines, 7 for methadone, 40 for buprenorphine, 106 for tramadol and 25 for MDMA. The specificity compared to the confirmation method was above 99% for all drug groups. The sensitivity was above 98% except for amphetamine (96%), MDMA (83 %), and buprenorphine (85%).

In the urine screening 536 were positive for THC, 287 for amphetamine, 63 for morphine, 171 for benzoylecgonine, 235 for benzodiazepines, 9 for methadone, 84 for buprenorphine, 147 for tramadol and 228 for MDMA (cross-reacts with amphetamine. The sensitivity in urine were above 99% for the screening. However, the specificity were between 94 to 100%, except for the MDMA assay which has a specificity of 83% due to the cross reactivity with amphetamine.

The discrepancies between blood and urine can be described as follows. For buprenorphine, seven samples screened positive in urine but negative in blood. The quantitative results were between the confirmation methods lower limit of quantification (0,2 ng/g) and the blood ELISA screening threshold of 1 ng/mL. For amphetamine, nine samples were screened positive in urine but negative in blood, however, all of these cases had a d-amphetamine concentration below 20 ng/ml which is the enantiomer specific for the blood screening. In total 227 positive hits in urine were not confirmed in blood which might be explained by the different detection times in blood and urine.

Conclusion/Discussion: In summary, the blood screening proved to be highly specific and also highly sensitive except for buprenorphine, where the threshold of 1 ng/mL was not low enough, and for MDMA were the blood screening failed to recognize five true positive samples. The urine screening, on the other hand, presented with numerous positive screening findings that could not be confirmed in blood. We conclude that blood was the best screening matrix to provide evidence for suspected driving under the influence of drugs if the drug needs to be confirmed in blood as well.

Keywords: Urine screening; blood screening; immunoassay, ELISA

Challenges and Insights in LC-MS/MS and LC/TOF-MS Analysis of Isobaric Compounds in the Opioid Class

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Background/Introduction: The current opioid epidemic and the involvement of fentanyl derivatives and other novel illicit opioids presents unique challenges during toxicological analysis. Constantly changing trends and the discovery of completely novel substances necessitates continuous updating and evaluation of the analytical method and scope used in their identification. Many of these substances, however, are either isomers or are structurally similar making their separation and quantitation more challenging. In developing public health responses, drug user education, harm reduction, and scheduling, enforcement, and interdiction it is crucial to be able to definitively identify these substances and to analytically differentiate them.

Objective: The objective of this study is to highlight analytical challenges and solutions associated with the detection of novel illicit opioids during toxicological analysis using specific casework examples.

Method: High Performance Liquid Chromatography/Time of Flight-Mass Spectrometry (LC/TOF-MS) is used in many laboratories for comprehensive drug screening applications, including novel drug identification. A limitation of LC/TOF-MS, which relies on identification of the compound by the accurate mass of the parent drug, includes the risk of not resolving isobaric compounds; further, structurally similar compounds may have very similar retention times. To address this, samples screening positive for these closely related drugs are confirmed and quantitated using High Performance Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS). During confirmation testing of novel illicit opioids, such as U-47700, carfentanil, and furanylfentanyl, investigations of the identity of potential interferents have led to the discovery of additional emergent substances and/or unknown metabolites of fentanyl related substances, prompting updating and expansion of the scope of testing in the confirmation panel.

Results: Examples of using these experiences to improve analytical scope include the identification of U-47700 which was first identified in our laboratory because it generated a screen-positive result with the correct exact mass and retention time of AH-7921 during the LC/TOF-MS screen. AH-7921 is a novel opioid agonist isobaric with U-47700. Upon confirmation, a peak with a shifted retention time was observed which was verified to be U-47700 after running the reference standard. After redeveloping a confirmatory LC-MS/MS panel to include U-47700, a second U-series compound, U-49900, was only identified when the reviewer noticed another chromatographic peak with the same transitions eluting close to the retention time of U-47700 in a series of five cases.

A third example is represented in an interference in the detection of the fentanyl analog methoxyacetylfentanyl. In 2017, methoxyacetylfentanyl began to be detected in many patient cases, which prompted development of a sensitive confirmation panel by LC-MS/MS. There was a high rate of unconfirmed methoxyacetylfentanyl positive LC/TOF-MS screens on the LC-MS/MS confirmatory method. Upon close review however, a shifted peak with the correct transitions was noted in the LC-MS/MS data. An investigation of other findings in those cases led to the theory that a metabolite of fentanyl, para-hydroxyfentanyl, which is an isomer of methoxyacetylfentanyl, was causing the false positive in the LC/TOF-MS screen. Shrinking the acceptable retention time window in the screen for methoxyacetylfentanyl and including a requirement to review the case for the presence of fentanyl, resulted in a significant decrease in false screen positives for this compound.

Chromatographic interferences have also been key in identifying additional opioids in patient cases including 3-methylfentanyl, and cyclopropylfentanyl.

Conclusion/Discussion: Analysis and differentiation of closely related and isobaric drugs in the opioid class requires careful scrutiny of all case data, awareness of the long list of possible isomers and isobars, careful validation, and regular updating of the scope of analysis. Analysts who review raw data should be attentive to and follow up on atypical out-of-scope findings such as interferences and unknown peaks, as they may be indicative of the presence of new designer opioids.

Keywords: Designer Opioids, Analysis, Challenges

Unique Structural/Stereo-Isomer and Similar-Mass Interference Analysis of Novel Fentanyl Analogues in Postmortem and DUID Whole Blood by LC─MS-MS

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Background/Introduction: The illegal manufacture and distribution of new fentanyl analogues (fentalogues) which share the same pharmacophore has created a significant analytical challenge for toxicology laboratories worldwide. Challenges include obtaining reference standards, optimizing instrumentation and methods to identify and quantify structural and geometric isomers, and distinguishing similar-mass analytes at lower concentrations. Interferences may be attributed to the presence of a structural analogue or stereoisomer, a similar-mass analyte, or a metabolite with identical fragment ions. Liquid chromatography tandem-mass spectrometry (LC-MS-MS) methods with short run times may misidentify these fentalogues.

Objective: Develop an LC─MS-MS method to identify and quantify novel fentalogues in 0.5 mL of whole blood.

Method: Sample preparation included osmotic lysing, precipitation with cold 10:90 methanol:acetonitrile solution and centrifugation using a Beckman Allegra™ 6 benchtop centrifuge with a GH-3.8 rotor at a relative centrifugal field (RCF) of 2,800 x *g*. Solid phase extraction was employed using the Strata® Screen-C (55µm, 70Å), 200 mg/6mL mixed-mode sorbent which incorporate the hydrophobic selectivity of a C8 phase and strong cation-exchange cartridge from Phenomenex (Torrance, CA). Analysis was performed by LC-MS-MS using a Thermo Scientific™ Vanquish™ Flex UHPLC system coupled with TSQ Quantis™ triple quadrupole tandem-mass spectrometer (Thermo Scientific, San Jose, CA). The mobile phase was composed of 0.1% formic acid (volume ratio) in water and 0.1% formic acid in acetonitrile. The column oven and autosampler were maintained at 40°C and 5°C, respectively. Chromeleon™ 7.2 SR4 and Thermo Xcalibur™ 4.1 softwares were utilized to control the LC. TSQ Quantis™ 3.0 SP1 and TraceFinder™ 4.1 SP3 softwares were used for data acquisition and analysis.

Results: The limit of detection (LOD) established for methoxyacetyl fentanyl, *para*-methoxyacetyl fentanyl, *para*-fluoroacryl fentanyl, fentanyl carbamate, 2-furanyl fentanyl (2-Fu-F), cyclopropyl fentanyl (CPF), crotonyl fentanyl, and carfentanil was 12.5 pg/mL. The LOD established for *N*-methyl norfentanyl, norfentanyl, norcarfentanil, 4-ANPP, acetylfentanyl, β-hydroxy fentanyl, benzyl fentanyl, acryl fentanyl, alfentanil, fentanyl, *para*-fluoro fentanyl (*p*-FF), (±)-*trans*-3-methylfentanyl, (±)-*cis*-3-methylfentanyl, butyryl fentanyl, *para/meta*-fluoroisobutyryl fentanyl (*p/m*-FIBF), sufentanil, phenyl fentanyl and cyclopentenyl fentanyl was at 62.5 pg/mL. Linear seven-point calibration curves with 1/x weighting in sheep blood were established between 0.025 – 4.0 ng/mL for analytes with the lower LOD and 0.125 – 20 ng/mL for analytes with the higher LOD.

Conclusion/Discussion: This sensitive method has been validated for whole blood according to the Scientific Working Group for Forensic Toxicology (SWGTOX) standard practices. Baseline resolution was achieved for the structural isomers CPF/crotonyl fentanyl, methoxyacetyl fentanyl/*para*-methoxyacetyl fentanyl/*para*-fluoroacryl fentanyl/fentanyl carbamate, and *cis*/*trans*-3-methylfentanyl/butyryl fentanyl. The *cis*- and *trans*- isomers of 3-methylfentanyl were also separated chromatographically along with the two similar-mass analytes of 2-Fu-F and cyclopentenyl fentanyl. Chromatographic separation was necessary for these isomers since most of them share the same quantifier and qualifier ions (188 and 105) and have similar ion ratios. This method also separates a common unknown peak which is suspected to be *para*-hydroxy fentanyl from methoxyacetyl fentanyl. Only chromatographic separation can distinguish this probable fentanyl metabolite from the parent fentalogue as both have the same molecular mass and fragment ions. The analyte retention times range from 4 to 18 minutes. Based on the developed method, extended run time may be necessary for the confident identification and quantification of these analytes.

Keywords: Fentanyl Analogues, cyclopropyl fentanyl, methoxyacetyl fentanyl

S50

Recent 5F-ADB Detection in Multiple U.S. Military Specimens by the Department of Defense New and Emerging Drugs Surveillance Program

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Background/Introduction: 5F-ADB (also known as 5-fluoro MDMB-PINACA) is a synthetic cannabinoid receptor agonist with an aminoalkylindazole structure and is sold as a cannabis substitute. This substance is mainly offered on the internet either in the form of herbal mixtures, where the chemical has been sprayed on plant material, as a powder, or in a liquid for vaporizing in e-cigarette devices. 5F-ADB was first identified overseas in 2014 from postmortem samples taken from individuals who had died after using a product called "Heart Shot BLACK". There is a recent case report regarding the suspected acute intoxication due to 5F-ADB in five individuals. Analytical information from this case report confirmed that the smoked material contained 5F-ADB, and urine specimens contained 5F-ADB metabolites.

Objective: We report 28 cases of analytically detected 5F-ADB 3,3-dimethylbutanoic acid, a major metabolite of 5F-ADB, in urine military specimens from a single military installation located in North Carolina.

Method: Urine specimens from 1,001 military service members were analyzed by LC-MS/MS among four screening panels -- a Synthetic Cannabinoid Panel containing 46 analytes (LOD 0.2 ng/mL), a Designer Drug Panel containing 75 analytes (LOD 1.0 to 5.0 ng/mL), a Hallucinogens and Stimulants Panel containing 45 analytes (LOD 0.25 ng/mL), and a Benzodiazepine Panel containing 36 analytes (LOD 1.0 to 10.0 ng/mL). The acceptance criteria for the identification of substances were based upon chromatography, retention times, ion ratios, and a single point calibration for comparison to LOD cutoff concentrations.

Results: In January 2018, approximately 60 service members with medical emergencies related to "vaping" products marketed as cannabidiol (CBD) oil, specifically called "Black Magic," presented at the emergency departments at Womack Army Medical Center at Ft. Bragg, NC and the Naval Medical Center at Camp Lejeune, NC. Symptoms ranged from headaches, nausea, vomiting, palpitations, dilated pupils, dizziness, confusion, disorientation, agitation, and seizures. Soon thereafter, 1,001 urine specimens from a military installation located in North Carolina were submitted to the Division of Forensic Toxicology, Armed Forces Medical Examiner System for LSD testing only, but none confirmed positive for LSD. The specimens were transferred to the Special Forensic Toxicology Drug Testing Laboratory (SFTDTL) Drug Surveillance Program for synthetic cannabinoid testing. The SFTDTL detected 28 urine specimens that contained 5F-ADB metabolite. This is a significant number of specimens with a 2.8% prevalence rate for an illegal synthetic cannabinoid from a very specific location, in distinction to the entire military population with an overall positive rate of less than 1% for drugs in 2017. No other synthetic cannabinoids were present.

Conclusion/Discussion: The correlation of multiple urine specimens containing a relatively new and dangerous synthetic cannabinoid with the numerous emergency department visits due to "vaping" is of great concern to the military, especially to personnel health and readiness of commands at Camp Lejeune and Ft. Bragg. This finding highlights the importance of new and emerging drug surveillance with large multiple-analyte testing panels. This situation resulted in a Public Health Alert within the U.S. Army, warning service members of the dangers of CBD oils and reminding soldiers that per Army Regulation 600-85, they are prohibited from using hemp or products containing hemp oil in addition to synthetic cannabinoids. Service members and the general population should be cognizant that "CBD oil" may not only contain CBD but also THC or synthetic cannabinoids such as 5F-ADB.

Keywords: 5F-ADB intoxication, Novel Psychoactive Substances (NPS), Surveillance testing

S51

The UNODC Drug Early Warning Advisory (EWA) Portal for the Collection of Toxicological Case Data on Emerging Drugs: Collaboration with the International Forensic Toxicology Community.

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Introduction: The United Nations Office on Drugs and Crime (UNODC) has established a drug early warning advisory (EWA) portal for the collection and sharing of toxicological case information focused on the rapid dissemination of information on emerging drugs, and novel psychoactive substances (NPS). The UNODC EWA Portal was established as an outcome of the Expert Consultation on Forensic Toxicology and Drug Control, held in Vienna, Austria, in June 2016. The consultation was organized pursuant to the Commission on Narcotic Drugs (CND) Resolution 58/7 of 2015, entitled "Strengthening cooperation with the scientific community, including academia, and promoting scientific research in drug demand and supply reduction policies in order to find effective solutions to various aspects of the world drug problem".

Objective: This presentation will introduce the audience to a new international collaborative resource for the timely sharing of toxicological data for emerging drugs.

PLATFORM

Method: An online portal was established by the UNODC for the entry of case information (https://www.unodc.org/tox/#/login). Following pilot exercises conducted to test the data collection form and the online platform, over 200 case reports have been submitted by laboratories in 17 countries. Collectively, the incidents in the system include over 220 reports on 80 substances associated with intoxications and deaths, including novel substances under international control, NPS and their metabolites.

Results: Toxicologists or institutions contributing data to the system will have real-time access to the aggregated data on the substances detected, region, date of report, methods of analysis used, sample type, drugs detected and their concentrations. One of the other main outputs of the EWA will be the publication of a threat assessment report that will be disseminated to contributors to the system. The report will give an overview of emerging threats, and identify NPS of greatest concern, based on their harmfulness, prevalence and persistence. At the international level, this report will support the NPS prioritization process of UNODC, which will feed into the information provided to the World Health Organization for its Expert Committee Meeting on Drug Dependence (ECDD) and inform national strategies. It will also enable the UNODC to better direct resources to laboratories which including the preparation of manuals, procurement of reference materials and preparation of test samples for proficiency programs.

Conclusion: In order to best support and ensure the sustainability of the portal, UNODC has reached out to the membership of professional groups such as TIAFT and SOFT to promote and support the advisory. TIAFT has committed to playing an integral role in the provision of data and review of material that is submitted for publication by the UNODC. It is envisioned that weekly alerts will be issued by the UNODC to users of the systems as well as other interested parties including police, clinicians and policy makers. These alerts will contain information on the type of NPS, where it was detected, the method of detection as well as other relevant toxicological information. This presentation will highlight the critical role of practicing toxicologists and their professional organizations in the success of the portal and its benefits to the community.

Keywords: Emerging Drugs, Database, United Nations

S52

Findings from DEA's National Forensic Laboratory Information System (NFLIS) Toxicology Laboratory Survey

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Introduction: The National Forensic Laboratory Information System (NFLIS) represents an important Drug Enforcement Administration (DEA) resource in monitoring illicit drug abuse and trafficking. Available NFLIS data reflect the results from drug chemistry analyses conducted by Federal, State, and local forensic laboratories across the country (NFLIS-Drug). NFLIS-Drug data are used to support drug scheduling decisions and to inform drug policy and drug enforcement initiatives nationally and in local communities around the country. The DEA is enhancing its efforts to combat diversion and identify new and emerging drugs of misuse and abuse by expanding NFLIS to include death data from medical examiner and coroner offices (MECs) operating in the United States (NFLIS-MEC) and drug testing–related data from toxicology laboratories (TLs) nationwide (NFLIS-Tox). The NFLIS-MEC and NFLIS-Tox data collections extend DEA's surveillance in identifying new and emerging drugs and to inform drug use patterns. In preparation for this expansion, DEA conducted a 2017 NFLIS Medical Examiner and Coroner Office Survey and a Toxicology Laboratory Survey.

Objective: The objective of this presentation is to present the results from the Toxicology Laboratory Survey which focused on four major topics, including laboratory identification, caseload and testing practices, information management system, and participation in a national data collection.

Method: The Toxicology Laboratory Survey was designed by NFLIS staff with the help of external consultants and based largely on the findings from the NFLIS Feasibility Study that was conducted in 2016 across 9 pilot site TLs and 9 pilot site MECs. Since there is no centralized repository for toxicology laboratories, the frame of laboratories was identified from multiple sources. Survey responses were collected via a mixed mode data collection (Web, mail, and telephone). Data collection began in June 2017 and concluded in October 2017. NFLIS staff performed several actions to increase survey responses including verification calls to confirm toxicology laboratory contact information and point of contact, prompting call reminders, and nonresponse calling to collect two critical questions. In addition, NFLIS staff reached out several organizations and outreach efforts to encourage participation.

Results: Survey results highlighting findings related to operation, caseloads, turnaround times, toxicology testing practices, accreditation, and information management systems of toxicology laboratories are presented.
Overall, a total of 231 out of 392 TLs completed the full survey for an overall response rate of 58.9%. Further, 68.1% provided responses to the critical items related to caseload information and types of toxicology testing services offered. Almost 6 in 10 (57%) TLs that responded to the survey were publicly funded while 43% were privately owned and operated. Of the 226 eligible respondents that answered the question pertaining to accreditation, 43% were accredited by the ANSI-ASQ National Accreditation Board, 36% were accredited by the Clinical Laboratory Improvement Amendments (CLIA), 26% were accredited by the College of American Pathologists (CAP), and 15% were accredited by the American Board of Forensic Toxicology (ABFT). TLs were asked to indicate their average turnaround time, in days, for completion of a toxicology case, excluding turnaround time for alcohol only cases. Across the 210 TLs responding to this question, the overall average turnaround time was 36.5 days.

Conclusion/Discussion: Attendees will gain a broad understanding of the operations, policies, practices, and resource needs of toxicology laboratories in the United States.

Keywords: National Forensic Laboratory Information System, Drug Enforcement Administration, Drug Surveillance

The Evolution of Military Drugs of Abuse: Surveillance and Testing

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Background/Introduction: In June 1971, President Nixon created the first military drug urinalysis program which was intended to identify marijuana and heroin use in military personnel returning from Vietnam. This eventually led to the formation of random drug testing in April 1974 to help deter drug use. Three decades later in 2013, synthetic cannabinoids and cathinones emerged on the drug scene, causing issues for civilian and military laboratories alike. As a result, a New and Emerging Drugs Surveillance Program was created. The goal was to provide information and identify trends with usage of novel and emerging psychoactive substances in the military. This would enable the Department of Defense (DoD) to make decisions and adapt drug-testing policies while maintaining military readiness.

Objective: To establish and maintain a surveillance program that relies on the most current knowledge of trends and actual testing of specimens from the military population.

Method: Four panels to screen for novel and emerging drugs were developed. The hallucinogens and stimulants panel analyzes 45 drugs with a variety of tryptamines, phenethylamines, and NMDA receptor antagonists. The designer drug panel scans for 75 drugs over a broad range of classes such as bath salts, fentanyl analogues, empathogen-entactogens, and hypnotic "ZZZ" drugs. The synthetic cannabinoids panel consisted of 46 of the most prevalent seized and observed drugs in this drug group. Finally, there was a panel of 36 benzodiazepines consisting of a mixture of legal and illegally synthesized drugs. The simple extraction schemes and sample loading on an ABSciex 4500 QTrap LC-MS/MS using multiple reaction monitoring (MRM) allowed for higher throughput and fast analytical run times for these methods.

Results: Since 2013**,** over 14,000 urine specimens have been tested by the Surveillance drug testing program. These were specimens that screened presumptively positive at the DoD Forensic Toxicology Drug Testing Laboratories but did not confirm positive by the limited mass spectrometry panels. DMAA is a banned substance for military personnel while phentermine, a legal substance, can be prescribed by military doctors, depending on the branch, for short-term use. However, each has been observed in surveillance testing at a steady rate of use, and were identified as the cause for increased rates of amphetamine immunoassay positives. Mitragynine is another banned substance that has been consistently seen in surveillance specimens. A variety of synthetic cannabinoids have been detected in surveillance specimens such as AB-PINACA N-COOH, JWH-073 N-COOH, and JWH-018 N-COOH. However, 5F-ADB, a new synthetic cannabinoid commonly found in vaping oils, has recently been detected in surveillance specimens. The most prevalent drugs detected from the designer drug panel are a stimulant known as D2PM and a cathinone called PV9. The hallucinogens and stimulants panel has detected ketamine/norketamine and fentanyl, although these drugs are also known to be part of emergency medical resuscitation practices. The benzodiazepines panel has shown consistent usage of clonazepam/7-aminoclonazepam and midazolam/alpha-hydroxymidazolam.

Conclusion/Discussion: Surveillance reports are generated every 2,000 specimens which tend to yield the largest observable changes in drug trends. With each iteration, the panels are re-validated and drugs are either added or dropped based on prevalence and scientific literature. However, the surveillance program allows for the flexibility to add new drugs in real-time for monitoring and determination of frequency. This policy has afforded the surveillance program a means to stay current and more proactive as opposed to reactive to new and emerging drug trends.

Keywords: Surveillance, Novel, Emerging

Degradation Of Synthetic Cathinones – Products Identification

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Background/Introduction: In recent years, a flood of synthetic cathinones (SC) has been observed. According to the European Drug Report 2018, published by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), synthetic cathinones were the second most frequently seized group of new psychoactive substances (NPS) in 2016. Together with synthetic cannabinoids, they cover over 70% of the NPS illicit market. The growing number of new compounds and their analogs or isomers, including existing substances, is in itself a challenge for the analyst toxicologist. Unfortunately, results of recent research show that not only the proper storage, but also a variety of environmental conditions prior to sample delivery to the laboratory should be taken into account. For some groups of cathinones, pH and especially temperature and storage time are crucial for their stability in biological material. In forensic laboratories, analysis of biological samples for the presence of these substances can sometimes be time sensitive. Therefore, these circumstances should be borne in mind when developing the method and interpreting analytical results.

Objective: The aim of the study was to detect and identify degradation products of 4-fluoromethcathinone (4-FMC, flephedrone) in water and blood samples, and to investigate the mechanism of the degradation process.

Method: Water and blood were analyzed for the presence of main degradation products of 4-FMC. At first, 4-FMC aqueous solution and a spiked blood sample were stored at room temperature up to 170 h. The samples were analyzed for degradation products every 24 h in the mass range m/z = 50 – 350 amu. The analytes were isolated from blood by acetone and acetonitrile precipitation. Samples were evaporated and reconstituted in mobile phase. Water samples were analyzed directly without treatment. Analyses were carried out using SCIEX 3200 QTRAP MS/MS and Agilent 6520 Accurate-Mass Q-TOF LC/MS. Positive ion detection was used.

Results: Two main degradation products of 4-FMC were detected and identified with parent ion masses observed at m/z = 205 and 219, respectively. The presence of sodium adduct (4-FMC-Na^+), characterized also by an ion with m/z = 205, was excluded. Analysis by LC-MS/QTOF determined the exact masses of the above compounds: 205.1128 Da and 219.1282 Da, respectively. Chemical formulas of the resulting products were thus proposed: $C_{12}H_{13}FN_2$ and $C_{13}H_{15}FN_2$, and in the next step potential structures of detected 4-FMC degradation products were identified. The results indicate that the process of 4-FMC decay in aqueous solution and in the blood is associated with the cyclization reaction leading most likely to the creation of 3-(4-fluorophenyl)-2,5-dimethyl-1,4-dihydropyrazine (2M-P) and 2-(4-fluorophenyl)-3,5,6-trimethyl-1,4-dihydropyrazine (3M-P), which are the products of 3,6-dimethyl-2,5-(4-difluorophenylodihydropyrazine) decomposition. The presence of 2M-P and 3M-P was detected in both the aqueous solution and in the blood sample spiked with 4-FMC. Surprisingly, there is no presence of 4-FMC cyclization product, i.e. 2,5-bis(4-fluorophenyl)-1,3,4,6-tetramethyl-1,4-dihydropyrazine (FMC-P-FMC), as it was documented in the earlier literature by Berrang, in case of cathinone.

Conclusion/discussion: The degradation of 4-FMC takes place in both the aqueous solution and in the biological material (blood). It has been confirmed by identifying two main decay products of the parent compound in both type of samples. No 4-FMC cyclization product, i.e. FMC-P-FMC, was observed, which may suggest its instability. The degradation products of unstable synthetic cathinones may, in addition to metabolites, be used as markers of the parent compounds in biological material.

Keywords: synthetic cathinones, degradation, LC-MS QTRAP


SOFT 2019
SAN ANTONIO, TX

P1

Comprehensive drug screening in whole blood samples using LC/Ion-Trap analysis and UCT QuEChERS extraction procedures.

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Background/Introduction: Over the past few years, there has been a steady increase in the number of police officers that are submitting DUI analysis requests with specific requests for drugs they suspect the driver may be under the influence of. These requests have increased dramatically after the sale of recreational marijuana in state licensed stores became legal on July 1st, 2017 in Nevada. The DUI testing policy for the forensic toxicology laboratory at the time was that any DUI whole blood sample with a BAC above 0.084% (taking uncertainty of measurement into account) would not go onto further testing to determine if there were any drugs the driver may have been under the influence of at the time of the incident. After a study on adjudicated whole blood DUI casework in late 2017, the Henderson Police Department's Forensic Toxicology Laboratory found that approximately 60% of the DUI cases from this study that were not previously analyzed due to having a BAC above 0.084% had drugs in their system at the time of the incident in which they were arrested for.

Objective: The aim of this work is to develop a rapid, robust and selective screening method that will allow for positive identification of specific drugs. Using a targeted screening method, the precursor ion scans will be filtered and analyzed by information dependent acquisition (IDA) data and enhanced product ion (EPI) scan experiments on an AB/Sciex Qtrap® 5500 instrument to readily identify each drug that screens positive in a whole blood sample.

Method: This method involves the rapid extraction of whole blood samples using the UCT QuEChERS extraction kits. The extraction is performed by adding 2 ml of acetonitrile/ammonium hydroxide (95:5) to the QuEChERS extraction tubes along with 1 ml of the whole blood sample and 100 µl of internal standard. The extraction tube is then vortexed to break up any salt agglomerates and centrifuged for 10 minutes at 2,500 rpm. 2 ml of the supernatant is then transferred to a UCT spin filter tube and that sample is centrifuged for 5 minutes at 5,000 rpm. Then 500 µl of the purified sample is transferred from the UCT spin filter tube to a clean glass tube. The purified sample is then evaporated at 35°C under nitrogen and reconstituted in 500 µl of HPLC-grade water/methanol (9:1). The analysis is performed on a Shimadzu Prominence UFLC coupled to an AB/Sciex Qtrap 5500 instrument. The analysis is performed with a phenyl-hexyl column (Phenomenex Kinetex, 50 x 4.6mm, 2.6 µm) at 40°C. The mobile phases consist of: (A) – 10 mM ammonium formate with 0.1% formic acid in water and (B) – 0.1% formic acid in methanol.

Results: The LC/Ion Trap screening method could readily detect and identify approximately 110 different substances in a 12-minute analysis. The method was validated using the parameters set forth in SWGTOX for validating a qualitative screening method on a LC instrument. The method showed great sensitivity, robustness and selectivity without the false positive and false negative results that were seen with other screening methods that were previously used in the forensic toxicology laboratories.

Conclusion/Discussion: The targeted drug screening method using the data dependent ion survey scans followed by the IDA and EPI experiments proved to be a very fast, selective and sensitive method in which to identify numerous drugs in a single analysis. The combination of the thorough analytical method coupled with the quick and easy extraction method allows for a significant decrease in the time it takes to screen whole blood samples for a large variety of drugs that a forensic laboratory may be interested in.

Keywords: Precursor ion scan, LC/Ion Trap, Drug Screening

POSTER

P2

Method Validation for Simultaneous Quantification of Fentanyl, Nor-fentanyl and Acetyl Fentanyl in Blood and Identification in Urine using LC-MS/MS.

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Introduction: Fentanyl is a potent synthetic opioid acting as a strong μ-receptor agonist with a potency 100 times that of morphine. As a result of its availability as a prescribed drug and its potency, it has been abused for decades. Additionally, clandestine laboratories have begun manufacturing fentanyl and its analog, acetyl fentanyl, leading to greater availability and misuse, often leading to overdose. A low therapeutic range makes it difficult to detect using conventional GC/MS methods, so a method was developed and validated using liquid chromatography tandem mass spectrometry (LC-MS/MS) to improve sensitivity.

Objective: The LC-MS/MS method will allow for the qualitative identification and quantitation of acetyl fentanyl, fentanyl, and fentanyl metabolite nor-fentanyl. Qualitative analysis is performed in urine. The drugs are extracted using CEREX Clin II solid phase extraction (SPE) columns and subsequently analyzed using LC-MS/MS dynamic multiple reaction monitoring. Isotopically labelled internal standards are used in the quantitative analysis of fentanyl, nor-fentanyl and acetyl fentanyl. The SWGTOX guidelines were followed for validations.

Method: Isotopically labelled internal standards were added to samples followed by 5 mL of pH 4.5 sodium acetate buffer. Samples were sonicated, centrifuged and applied to Cerex Clin II SPE columns preconditioned with ethyl acetate and methanol. The columns were washed with potassium carbonate buffer pH 9 and deionized water, before elution with ethyl acetate and ammonium hydroxide at 98:2 ratio. Samples were reconstituted with mobile phase (90% water/0.1% formic acid, 10% LC/MS grade methanol) and transferred to auto sampler vials before injecting onto an Agilent 1200 series LC coupled to an Agilent 6410 Triple Quadrupole Mass Spectrometer. HPLC separation was attained by using water with 0.1% formic acid and a gradient of methanol over an Agilent Zorbax C18 HPLC column (1.8 μM X 2.1 x 50 mm) at 50º C and analyzed using dynamic multiple reaction monitoring. Validation was performed using SWGTOX guidelines for calibration model fits, accuracy and precision, sensitivity measured by the limit of detection (LOD) and limit of quantitation (LOQ), recovery and efficiency, carryover, interference, ion suppression/enhancement, dilution integrity and stability.

Results: All analytes were determined to fit to a linear 1/x weighted curve with an $R^2 \geq 0.999$ with an LOQ of 1 ng/mL and an upper limit of quantitation of 100 ng/mL. The LOD of Fentanyl, Nor-Fentanyl and Acetyl Fentanyl in blood is 0.12ng/ml, 0.41ng/ml and 0.25 ng/ml, respectively. The LOD for urine is 1ng/ml.

Percent accuracy at three concentrations ranged from 0.44-4.35%, between run bias %CV of 1.52-3.18% and within run bias %CV of 2.67-5.65%. All of the numbers are within acceptable CV values of ± 20 %. No significant carryover, interference from matrix effects or drugs of abuse, or ion suppression/enhancement effects were noted. Furthermore, samples proved stable over 5 days and were successfully quantitated at dilutions of 1:2, 1:4 and 1:10.

Conclusion: A method for the quantitation of fentanyl, nor-fentanyl and acetyl fentanyl in postmortem and ante mortem blood, and confirmation in urine was successfully developed and validated using SPE and LC/MS-MS. The sensitivity of this method allowed us to successfully confirm and quantitate fentanyl, nor- fentanyl and acetyl fentanyl that were routinely not seen by GC/MS in our laboratory. Additionally, this method was developed using a methanol mobile phase instead of acetonitrile which has a large cost advantage.

POSTER

P3

Withdrawn

P4

Development of a LC-MS/MS Method for the Quantitation of Non-fentanyl Opioids

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Background/Introduction: Synthetic opioids represent an important class of drugs in toxicology labs as they are subject to abuse and may lead to impairment or contribute to death. For example, methadone which is commonly used in opioid substitution therapy may cause respiratory depression. Additional opioids, such as tramadol and tapentadol, have been approved for the treatment of moderate to severe pain and may also result in respiratory depression. Consequently, a robust method for detection of non-fentanyl opioids in human fluid and tissue samples is essential.

Objective: The aim of this study was to develop a robust method for the extraction and LC-MS/MS quantitation of methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), tramadol, N-desmethyltramadol, O-desmethyltramadol, tapentadol, and N-desmethyltapentadol from various matrices for postmortem and DUID testing.

Method: Nine calibrators spanned the range of 10 ng/mL to 2000 ng/mL. The QCs were comprised of low (30 ng/mL), mid (800 ng/mL) and high (1600 ng/mL) levels. Isotopically labeled internal standards were also utilized. To 0.5 mL of sample, 0.5 mL of saturated sodium borate buffer (pH 12) was added, followed by addition of 2.0 mL of ethyl acetate. The samples were mixed for 10 minutes. After centrifugation, the organic layer was transferred to a clean tube and dried down at room temperature using nitrogen. The samples were reconstituted in 2.0 mL of 80:20 (v:v) 7 mM ammonium formate + 0.05% formic acid in water: acetonitrile.

Extracted samples were separated using an Agilent 1200 series HPLC equipped with an Agilent ZORBAX Eclipse Plus C18 (3.0 x 100 mm, 3.5 µm) column. The HPLC was coupled to an Agilent 6460 triple quadrupole mass spectrometer for analysis. Mobile phase A consisted of 7mM ammonium formate + 0.05% formic acid in water, and mobile phase B was acetonitrile. The mass spectrometer was operated using dynamic MRM in positive ion mode and monitored two transitions per analyte.

Results: The optimized LC separation consisted of an initial isocratic hold at 20% B for 3.2 minutes, followed by a linear increase from 20-55% B in 0.1 minutes, 55-60% B in 1.2 minutes, and 60-95% B in 2 minutes. The column was held at 95% B for 1 minute prior to re-equilibration at 20% B. A flow rate of 0.40 mL/min was used, and the column was maintained at 40 °C. The initial isocratic hold resulted in separation of the structurally similar drugs tramadol and tapentadol as well as their desmethyl metabolites. The retention time, parent ion and fragment ions for the analyzed drugs and their metabolites are listed in the following table.

Analyte	Retention Time (min)	Parent Ion (*m/z*)	Fragment ions (*m/z*)
O-desmethyltramadol	1.94	250.2	58.1 and 232.2
Tramadol	3.99	264.2	58.2 and 246.2
N-desmethyltapentadol	4.10	208.2	107.0 and 121.1
N-desmethyltramadol	4.15	250.2	44.2 and 232.3
Tapentadol	4.41	222.2	107.1 and 121.1
EDDP	5.85	278.2	186.1 and 234.1
Methadone	6.04	310.2	105.0 and 265.1

Conclusion/Discussion: An extraction and LC-MS/MS method for the quantitation of non-fentanyl opioids was successfully developed. This method is capable of separating several opioids and their metabolites in less than 6.5 minutes. Baseline separation was achieved for the isomeric metabolites O-desmethyltramadol and N-desmethyltramadol. In addition, O-desmethyltramadol was baseline separated from its parent drug, tramadol. While tapentadol and its metabolite N-desmethyltapentadol displayed nearly identical fragmentation patterns as a result of their structural similarity, they were sufficiently differentiated by their retention times and precursor ions. This method will be validated in accordance with SWGTOX and ABFT guidelines.

Keywords: opioids, LC-MS/MS, method development

P5

Application of High Resolution Mass Spectrometry for Fentanyl Analog Confirmation and Metabolite Discovery in Forensic Urine Casework

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Introduction: Illicit availability and use of fentanyl and its analogs is increasingly evident across medical examiner toxicology findings; consequently, validated methods for detection and confirmation of these substances is essential for medical examiner as well as other court-related casework.

Previously we have described the use of high resolution mass spectrometry (HRMS) as the confirmatory technique in a dual-definitive screening and confirmation protocol for urine. This workflow has already been validated for forensic identification of many illicit and pharmaceutical drugs, and their metabolites, in court-related casework, however an expanded panel to incorporate newer fentanyl analogs is now required. HRMS using time-of-flight with multiple reaction monitoring (TOF-MRM) provides the enhanced sensitivity required for confirmation of low level fentanyl and analogs, while TOF-MS^E provides an additional analytical tool for the discovery of potential analog metabolites in human urine.

Objective: The objective of this analytical work was to develop a UPLC-HRMS method for confirmation of fentanyl along with its analogs. A previously described novel procedure, threshold accurate calibration (TAC) technique, was utilized for matrix-effect normalization. An additional aim was to perform further HRMS analysis of de-identified case samples for analog metabolite profiling based upon common exact-mass fragment ions and metabolic reactions modeling with Waters UNIFI software.

Method: Initial screening for acetylfentanyl, acrylfentanyl, 4-ANPP, butyrylfentanyl, isobutyrylfentanyl, carfentanil, cyclopropylfentanyl, fentanyl, fluorobutyrfentanyl, fluoroisobutyrfentanyl, furanylfentanyl, β-hydroxyfentanyl, methoxyacetylfentanyl, 3-methyfentanyl and norfentanyl was performed by dual-transition UPLC-MS/MS analysis using a Waters ACQUITY UPLC I-Class (FTN) system with a 3.3 minute chromatographic separation on an ACQUITY UPLC® BEH Phenyl column (1.7 µm, 2.1 x 50 mm, Waters) interfaced by electrospray (ESI) with a Waters Xevo TQD tandem mass spectrometer under MassLynx software control. Confirmation testing was by dual-ion UPLC-TOF-MRM, and employed Waters ACQUITY UPLC I-Class separation on an ACQUITY HSS C18 analytical column (1.7 mm, 2.1 x 150 mm) interfaced by ESI with a Waters G2-XS QTOF mass spectrometer using UNIFI Scientific Information System. The TAC technique was employed for both screening and confirmation i.e., sample preparation used dual aliquots (50 µL) of threshold calibrator, controls and case specimens; addition of reference analytes to the spiked wells and injection recovery standard (methapyrilene) to neat and spiked wells was followed by dilution (total 1:13) followed by analysis of all samples. Metabolite profiling was performed by UPLC-TOF-MS^E analysis with full MS acquisition at low and ramped collision energies.

Results: UPLC-TOF-MRM performance data for 10 analytical runs was used to assess accuracy and precision. Quality control performance for fentanyl and analogs was evaluated for analytical recovery at 0.8 ng/mL (85-103% recovery), 1.0 ng/mL (87-97%), 2.5 ng/mL (97-109%), 3.0 ng/mL (98-104%) and 10 ng/mL (98-118%). Precision at 0.8 ng/mL (10-22% CV), 1.0 ng/mL (8-19%), 2.5 ng/mL (7-16%), 3.0 ng/mL (11-16%) and 10 ng/mL (11-18%) was also within validation criteria. Confirmatory testing for 20 cases, that initially screened positive, demonstrated concordant findings for fentanyl (14 cases), norfentanyl (15), 4-ANPP (9), β-hydroxyfentanyl (7), acetylfentanyl (5), para-fluoroisobutyrfentanyl (4), methoxyacetylfentanyl (3) and furanylfentanyl (2).

TOF-MS^E analysis with metabolite profiling software revealed both major and minor metabolites for the fentanyls and 4-ANPP, based upon dealkylation, hydroxylation, acetylation and phase two metabolite pathway profiling. Confirmation of newly proposed metabolites awaits the availability and analysis of reference metabolite standards.

Conclusions: Screening (UPLC-MS/MS) and confirmatory (UPLC-TOF-MRM) methods for a panel of fentanyl analogs along with 4-ANPP, fentanyl and norfentanyl were developed and validated for use in forensic casework. Additional metabolite profiling by UPLC-TOF-MS^E provided insight into potential metabolites in authentic urine samples and revealed common fragment ions of potential value in the detection of metabolites, as well as newly emerging fentanyl analogs.

Keywords: Urine Drug Screening and Confirmation, Fentanyl Analogs, TOF-MS^E

POSTER

P6

Synthesis, Characterization and Certification of Barbarin, a Possible Botanical Source of Aminorex Identifications

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Background: Aminorex ((*RS*)-5-phenyl-4, 5-dihydro-1, 3-oxazol-2-amine) is a DEA Schedule I controlled substance that was marketed as an appetite suppressant but withdrawn when it was found to cause deaths from pulmonary hypertension. Aminorex~~, however,~~ has also been identified in human and equine forensic urine samples as a metabolite of levamisole, a veterinary anthelmintic and immunostimulant that is metabolized in mammalian systems, including humans, to aminorex. Multiple findings for aminorex in blood and urine samples of horses have been attributed to levamisole exposure due to the presence of other levamisole metabolites in these samples. However, several recent confirmed equine findings of aminorex did not include any evidence for levamisole exposure, suggesting exposure of the horses to other sources of aminorex. Preliminary evaluations suggest that the glucosinolate glucobarbarin may be a source of urinary aminorex in horses. Glucobarbarin is found in plants of the genus Barbarea in the Brassicaceae family. In these plants Glucobarbarin is hydrolyzed by myrosinase to an intermediate which cyclizes to barbarin, an insect repellant or attractant when the relevant plant structure is damaged. Glucobarbarin or barbarin are potential plant sources of equine aminorex identifications and we now report the synthesis of barbarin as a reference standard for research related to botanical sources of aminorex in equine forensic samples

Objective: The objective of this project was to synthesize and characterize barbarin for use as a reference standard in barbarin related research and to synthesize sufficient quantities of barbarin to perform oral administration studies to assess its disposition.

Method: Barbarin was synthesized by a condensation reaction starting with 2-hydroxy-2-phenylethylamine in THF by addition of excess carbon disulfide in the presence of pyridine. The resultant mixture was refluxed for 16 hours and the progress of the reaction monitored by TLC. When the reaction was complete, the reaction mixture was cooled and concentrated under vacuum; the resultant mixture was dissolved in DCM, washed with HCl, the aqueous portion extracted again with DCM. The organic layers were combined, dried with magnesium sulfate, and the yellow solid recrystallized twice from a DCM/hexanes mixture to provide a white, crystalline solid.

The structure was confirmed by ^{1}H-NMR, ($CDCl_3$, 400 MHz) δ (ppm): 7.62 (br s, 1H), 7.35-7.45 (m, 5H), 5.87 (t, 1H), 4.15(dd, 1H). 3.74(dd, 1H). Combustion analysis (CHNS, Calc: C, 60.31%, H, 5.06%, N7.81%, S, 17.89%. Found: C 60.16%, H 4.97%, N 7.77%, S 17.89%. High resolution MS. (ESI, M+1) found: $C_9H_{10}NOS$ 180.0478. Spectroscopic purity was greater than 99.5% by reversed-phase HPLC UV detection (diode array).

Results: A useful synthetic methodology for the plant toxin barbarin has been developed. Using this method barbarin has been successfully synthesized, purified and characterized. Barbarin is now available in small quantities as a certified reference standard for analytical/forensic toxicological research. Additionally this synthetic method is fully capable of producing larger quantities of barbarin if required for equine administration experiments.

Discussion/Conclusion: Aminorex has been identified in human and equine forensic urine samples as a metabolite of levamisole. More recently, however, aminorex has been identified in equine urine samples without any evidence for exposure to levamisole. One hypothesized source of equine urinary aminorex identifications is metabolic transformation of barbarin following exposure to plants of the Brassicales family. Gram quantities of chemically pure barbarin made possible by this research project will allow more in-depth investigation of the potential role of glucobarbarin and or barbarin as a chemical precursor of aminorex in horses and perhaps in other mammalian metabolic systems. In support of this hypothesis, preliminary administration experiments in which horses were administered Brassica vulgaris plant material showed evidence for the presence of aminorex in the four and eight hour post administration urine samples.

Keywords: Synthesis, Barbarin, Aminorex

POSTER

P7

Updates from National Forensic Laboratory Information System (NFLIS): 2017 Midyear Report

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Introduction: The National Forensic Laboratory Information System (NFLIS) represents an important Drug Enforcement Administration (DEA) resource in monitoring illicit drug abuse and trafficking. Available NFLIS data reflect the results from drug chemistry analyses conducted by Federal, State, and local forensic laboratories across the country (NFLIS-Drug). The NFLIS-Drug participation rate, defined as the percentage of the national drug caseload represented by laboratories that have joined NFLIS, is currently over 98%. This includes 50 state systems and 102 local or municipal laboratories/laboratory systems, representing a total of 280 individual laboratories. NFLIS-Drug data are used to support drug scheduling decisions and to inform drug policy and drug enforcement initiatives nationally and in local communities around the country.

Objective: The objective of this presentation is to present the results of drug cases *submitted* to State and local laboratories from January 1, 2017, through June 30, 2017, which were *analyzed* by September 30, 2017. The data presented include *all* drugs mentioned in the laboratories' reported drug items.

Method: National estimates of the top 25 drugs submitted to State and local laboratories are presented. Semiannual national trends for the estimated number of prescription drug reports that were identified as fentanyl, alprazolam, oxycodone, hydrocodone, buprenorphine, and amphetamine are presented. Major drug categories such as synthetic cannabinoids and narcotic analgesics are also presented. Selected drugs or drug categories by regional trends are presented. As available, we will update presented data for drug cases *submitted* to State and local laboratories from January 1, 2017, through December 30, 2017, which were *analyzed* by March 30, 2018.

Results: From January 1, 2001, through June 30, 2017, an estimated 452,380 distinct drug cases were submitted to State and local laboratories in the United States and analyzed by September 30, 2017. From these cases, an estimated 776,836 drug reports were identified. Cannabis/THC was the most frequently reported drug (174,077), followed by methamphetamine (170,300), cocaine (112,756), and heroin (78,833). These four most frequently reported drugs accounted for approximately 69% of all drug reports. Nationally, fentanyl reports remained steady from 2001 to 2005, followed by a noticeable increase in 2006 and dramatic increases from 2014 to 2017 ($p < .05$). Alprazolam reports showed a steady increase from the second half of 2003 through the first half of 2010, increased significantly again from 2014 to the first half of 2016, then decreased through 2017. Oxycodone and Hydrocodone reports generally decreased after 2010 through the first half of 2017. Buprenorphine reports showed a dramatic increase from 2013 to 2017. Amphetamine reports steadily increased between 2007 and 2017. For methamphetamine reports, all regions showed increases beginning around 2010 and 2011 and continuing through the first half of 2017, except that the West region had a decrease in reports from the first half of 2016 to the first half of 2017. For cocaine reports, all four regions had rolling decreasing trend lines with slight increases in the first half of 2017. FUB-AMB and 5F-ADB accounted for 65% of all reported synthetic cannabinoids during the first 6 months of 2017. Fentanyl, oxycodone, and hydrocodone accounted for 63% of narcotic analgesic reports during this period.

Conclusion/Discussion: Attendees will gain an understanding of NFLIS-Drug as a comprehensive information system that includes data from forensic laboratories that handle the Nation's drug analysis cases. NFLIS publicly shares aggregated and analyzed data through various reports throughout the year including national and regional trends of drugs submitted to State and local laboratories, as present here. These publications can benefit crime laboratory managers by increasing their awareness of emerging substances and other drug trends.

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

POSTER

P8

Oxcarbazepine Overdose in a Polysubstance Related Suicide

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Background/Introduction: Oxcarbazepine is a derivative of carbamazepine that is used primarily in the treatment of epilepsy, and experimentally as a mood-stabilizer in adjunctive therapy for the treatment of bipolar disorder. Oxcarbazepine is converted through oxidation to its pharmacologically active metabolite 10-OH-Carbazepine, which is thought to be responsible for most of the anti-convulsant action of the drug. Adverse effects of oxcarbazepine are generally dose-dependent and may include fatigue, somnolence, dizziness, diplopia, nystagmus, and ataxia. The therapeutic range for oxcarbazepine is based on the metabolite and extends from 6-35 mcg/mL. Toxicity has been reported with 10-OH-Carbazepine levels as high as 65 mcg/mL, and one fatality has been documented with a 10-OH-Carbazepine concentration of 92 mcg/mL.

Hydrocodone is a narcotic analgesic that undergoes demethylation and reduction to produce several pharmacologically active metabolites, including hydromorphone, norhydrocodone, and dihydrocodeine (6-α-hydrocodol), which contribute to its efficacy. Hydrocodone toxicity may be characterized by respiratory depression, drowsiness, and coma. Therapeutic blood and plasma concentrations of hydrocodone typically range from 10-50 ng/mL, while levels greater than 100 ng/mL are considered toxic, and concentrations exceeding 200 ng/mL are potentially fatal.

Diazepam is a benzodiazepine known for its efficacy and rapid onset. Therapeutic ranges of diazepam and its metabolite nordiazepam in blood and plasma measure between 200-2500 ng/mL. Diazepam toxicity may result in drowsiness, weakness, ataxia, and coma; however, serious and fatal effects are uncommon with diazepam if used singularly. Most terminal adverse events associated with diazepam are the result of interaction or combination with other drugs, especially CNS depressants.

Objective: This case presents a polysubstance related suicide involving the synergistic effect of toxic concentrations of oxcarbazepine and hydrocodone, in combination with the presence of diazepam. The concentration of 10-OH-Carbazepine found in the decedent's blood is the highest found in literature.

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Methods: Routine screening of postmortem blood was done using Enzyme Multiplied Immunoassay Technique (EMIT) and liquid-liquid alkaline extraction followed by gas chromatography/mass spectrometry (GC/MS) analysis. 10-OH-Carbazepine and hydrocodone and its metabolites were quantified by an external laboratory using liquid chromatography/tandem mass spectrometry (LC-MS/MS). Diazepam and nordiazepam quantitation was performed using high performance liquid chromatography (HPLC).

Results: The decedent is a 67-year-old female with a history of depression, psychiatric hospitalization, and previous suicide attempts. The decedent was found lying supine in bed with a bottle of hydrocodone in one hand and a can of soda in the other, next to a suicide note. Several other prescription medications including oxcarbazepine, gabapentin, diazepam, quetiapine, tizanidine, and lorazepam were found at the scene. Analysis of postmortem blood confirmed the presence of 10-OH-Carbazepine at 180 mcg/mL, hydrocodone at 490 ng/mL, and diazepam at 465 ng/mL.

Conclusion/Discussion: The most significant finding in this case is the 10-OH-Carbazepine concentration of 180 mcg/mL, which is greater than the highest known fatal level of 92 mcg/mL. The cause of death in this case was ruled oxcarbazepine and hydrocodone intoxication with diazepam use, and the manner of death was suicide.

Keywords: Oxcarbazepine, overdose, fatality

P9

Quantitative Analysis of Novel Synthetic Opioids, Morphine, and Buprenorphine in Oral Fluid by LC-MS/MS

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Background/Introduction: The opioid epidemic has become a national health emergency in the United States. While heroin and prescription opioid abuse is not uncommon, synthetic opioid use has risen dramatically, creating a public safety concern. Like traditional opioids, novel synthetic opioids are abused due to their analgesic and euphoric effects. Some adverse side effects include respiratory distress, nausea, and decreased consciousness. Synthetic opioids have emerged into the illicit and online drug market, including AH-7921, MT-45, U-series, and W-series. Though originally developed by pharmaceutical companies, these substances are not well studied in humans and comprehensive analytical methods for detecting and quantifying these opioids are limited. Oral fluid is a useful biological matrix for determining recent drug use, does not require a trained medical professional, and can be collected under direct observation, deterring adulteration.

Objective: The purpose of this research was to develop and validate a comprehensive analytical method for the detection and quantification of morphine, 6-acetylmorphine (6-AM), buprenorphine, U-47700, U-49900, U-50488, AH-7921, MT-45, W-18, and W-15 in oral fluid collected via Quantisal.

Methods: Analytes were isolated from oral fluid by solid-phase extraction and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Deuterated internal standard solution (25 µL of mixed 1000 ng/mL methanolic solution) was added to 1 mL of a buffer/oral fluid mixture (750 µL Quantisal buffer, 250 µL drug-free oral fluid) to achieve a final concentration of 100 ng/mL. Calibrator or QC solutions (25µL) were added, followed by 2 mL of 0.1M phosphate buffer (pH 6). Samples were vortexed, loaded onto PolyChrom ClinII 3 cc (35 mg) solid phase extraction (SPE) columns, and allowed to flow under gravity. The columns were rinsed with deionized water (1 mL) and 1 M acetic acid (1 mL). Cartridges were dried under 20 psi nitrogen for 5 min then washed with 1 mL hexane. Acidic drugs were eluted using ethyl acetate (1 mL). Following a 1 mL methanol wash, basic drugs were eluted using 1 mL of dichloromethane:isopropanol:ammonium hydroxide (80:20:5) then evaporated under nitrogen at 50ºC. The samples were reconstituted in 1 mL of 0.05% formic acid and 5 mM ammonium formate in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B) (95:5). This method was validated according to the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines. Oral fluid specimens were collected via Quantisal devices from 18 anonymous detainees in a Texas adult detention center in accordance with a Sam Houston State University Institutional Review Board (IRB) approved protocol

Results: The limits of detection and quantification were 5 ng/mL and 10 ng/mL, respectively. Linearity was observed between 10 and 500 ng/mL (R^2>0.99). Bias and imprecision were <±11.1%. Matrix effects ranged from −21.1 to 13.7%. No carryover was detected following injection of the highest calibrator (500 ng/mL). All analytes were stable (<±15% change from baseline) under all tested conditions (24 h room temperature, 72 h at 4 °C, and in the autosampler for 60 h at 4 °C). Oral fluid was collected from 18 adults: 9 males (24-42 years) and 9 females (23-47 years). From the authentic samples analyzed, morphine was detected in 4 cases and 6-AM was detected in 3 of those 4 subjects. Morphine concentrations were <LOQ, 32, 104, and 146 ng/mL and 6-AM concentrations in those same individuals were <LOD, 15, <LOQ, and 110 ng/mL.

Conclusion/Discussion: A comprehensive method for the quantification of morphine, 6-AM, buprenorphine, U-47700, U-49900, U-50488, AH-7921, MT-45, W-18, and W-15 in oral fluid was optimized and met SWGTOX validation criteria**.** To our knowledge, this is the first publication with a validated method for extraction and quantification of W-series drugs. The extraction technique and LC-MS/MS parameters can also be easily translated to other biological matrices.

Keywords: Novel Synthetic Opioids, Oral Fluid, LC-MS/MS

POSTER

P10

Comparison between two Liquid Chromatography-Tandem Mass Spectrometric Methods for Immunosuppressive Drugs in Whole Blood

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Background/Introduction: Therapeutic drug monitoring of immunosuppressive drugs is a key application in the toxicology laboratory. Currently, cyclosporine A, tacrolimus and sirolimus are among the main drugs used to guide therapy after organ transplantation, and liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the preferred technique for their analysis. Despite the widespread use of LC-MS/MS, there is still a need of fully validated methods for the analysis of immunosuppressive drugs. LC-MS/MS methods that are not validated can perform poorly resulting in inaccurate concentrations of drugs with potential clinical impact. Our laboratory has been analyzing cyclosporine A, tacrolimus and sirolimus by LC-MS/MS for over ten years. A new LC-MS/MS method was recently developed to replace the established one. Both methods use the same sample preparation procedure, but different chromatography. In the new method, a 3200 quadrupole linear ion trap (QTRAP) mass spectrometer with an electrospray ionization ion source is used, whereas the established method uses a 3000 QTRAP instrument with an atmospheric pressure chemical ionization ion source.

Objective: The goals of this study were (1) to assess the analytical performance of the new method with emphasis on accuracy and precision at low concentrations of drugs, 25 ng/mL for cyclosporine A, and 1 ng/mL for sirolimus and tacrolimus, and (2) to compare the results of the new method with the established one, and evaluate whether the methods can be used interchangeably for therapeutic drug monitoring.

Method: Immunosuppressive drugs were extracted from blood by protein precipitation using aqueous zinc sulfate and acetonitrile. The sample extracts were separated by liquid chromatography (Table 1). The new method was validated following the guideline of the Scientific Working Group for Forensic Toxicology (SWGTOX). Accuracy and intra-assay precision were evaluated following criteria for therapeutic drug monitoring as recommended by the International Association of Therapeutic Drug Monitoring and Clinical Toxicology Immunosuppressive Drug Scientific Committee. In method-comparison studies, 322 clinical samples were analyzed with the new method, and results were compared with those obtained by the established procedure. Bias and precision statistics were estimated using Passing-Bablok regression and Bland-Altman analysis.

Table 1. Chromatographic Conditions

	New Method	Established Method
Column	Halo Peptide ES-C18 (100x4.6 mm, 5 µm)	Zorbax SB-C18 (75x4.6 mm, 3.5 µm)
MPA	2 mM CH_3COONH_4 / 0.1% HCOOH/ H_2O	15 mM CH_3COONH_4 /0.5% CH_3COOH/ H_2O
MPB	2 mM CH_3COONH_4 / 0.1% HCOOH/ CH_3OH	CH_3OH
Time (min)/ MPA (%)/ Flow (mL/min)	0–0.1/65/0.8; 0.1–1/65/1; 1–3.8/5/0.8; 3.8–4.9/65/1; 4.9–5/65/0.8	0–1.5/100/1.6; 1.8–3.5/0/1.6; 3.5–4.2/100/1.6

Results: Accuracy and intra-assay precision were within -5.5%/+0.9%, and 1.1%/9.8%, respectively, throughout the calibration ranges, 25–2000 ng/mL for cyclosporine A, and 1–40 ng/mL for sirolimus and tacrolimus. Absolute matrix effects varied from 87% to 97%, and the relative matrix effect was ≤12%. Using the therapeutic interval, the results from both methods agreed in 96.6% of the cases. For clinical samples with either a sub-therapeutic or a supra-therapeutic concentration in one method and within the therapeutic range in the other, the results from either method could be used to adjust dosage to the same extent, and thus not expected to negatively affect patient care in clinical practice.

Conclusion/Discussion: An LC-MS/MS method for cyclosporine A, sirolimus and tacrolimus was validated following the SWGTOX guideline, and validation results met requirements for therapeutic drug monitoring of immunosuppressive drugs. In method-comparison studies, the bias between the new and the established method was not statistically significant. More importantly, clinical results correlated well and thus are not expected to adversely affect clinical decisions regarding dosing. This work highlights the importance of establishing the fitness-for-purpose of new methods before implementation in clinical practice.

Keywords: LC-MS/MS, Method Validation, Therapeutic Drug Monitoring

POSTER

P11

Detection of 3-methoxyphencyclidine in Cohort of Emergency Room Patients

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Background/Introduction:

In a four-week time span, sixteen patients (10 male, 6 female, ages 18-66) were seen though the emergency center who screened positive for PCP in urine via immunoassay; however, samples were negative for PCP upon confirmation. The policy of our laboratory is to investigate false positive screens as far as we can to attempt to elicit an explanation for the result. With the significant increase in novel psychoactive substances that have variable reactivity with current immunoassays, we have found this is one way to identify NPS's and provide as much information to physicians as possible.

Objective: To investigate the cause of multiple false positive immunoassay screens for PCP, as well to correlate the clinical signs and symptoms of the patients who were found to be positive for 3-MeO-PCP.

Method: Urine samples were collected from all patients. Standard drug screens were performed, which included immunoassay screens with automatic reflex to confirmation testing. Immunoassay screening was performed on an Abbott Architect c4000 with a PCP cut-off of 50 mg/dL. Opiate confirmation was performed on Waters TQS LCMSMS. Cocaine and PCP confirmations were performed on Agilent 5975 or 5977 GCMS in SIM mode. For any unconfirmed confirmation result, it is the policy of the laboratory to make every attempt to discover the cause of the negative confirmation test. For the negative PCP confirmations, a basic-acid-neutral extraction was performed and analyzed on an Agilent 5975 or 5977 GCMS in full scan mode.

Results: 3-MeO-PCP was confirmed in urine from all sixteen patients. In addition, fentanyl, cocaine and/or 6-MAM were also confirmed in all patients (15 positive for fentanyl, 11 positive for cocaine, 11 positive for 6-MAM). Upon reviewing the patients' charts, there was no indication of any patient who exhibited the classic signs and symptom of PCP ingestion.

Conclusion/Discussion: Phencyclidine (PCP) was introduced in the 1950s as an anesthetic and subsequently withdrawn from the market in the 1960s due to hallucinogenic side effects. During the following decades, recreational use of PCP skyrocketed but has since fallen out of favor and is among the less commonly encountered drugs of abuse. While PCP is currently a Schedule II compound in the United States, new novel versions such as 3-methoxy PCP (3-MeO-PCP) and 4-methoxy PCP (4-MeO-PCP) now exist for research and illicit purposes. Neither is currently scheduled by the DEA. There are very few published reports of 3-MeO-PCP, but those that have indicated the symptoms include dissociation, hallucination, and delirium. 3-MeO-PCP was confirmed in 16 emergency room patients. Reviewing the charts of all patients revealed nearly all patients did not exhibit the expected clinical signs and symptoms of 3-MeO-PCP ingestion. The only other common drug found in all but one patient was fentanyl, though all patients also confirmed positive for cocaine and/or morphine. With the lack of reported clinical signs, the conclusion was that 3-MeO-PCP was used as an additive/cutting agent to the fentanyl at an amount that was insignificant to the user.

Keywords: 3-methoxyphencyclidine, Emergency Room, Novel Psychoactive Substance

POSTER

P12

Fast and Simple Creatinine Determination in Wastewater by Liquid Chromatography-Mass Spectrometry

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Background/Introduction: Wastewater-based epidemiology is a fast and cost-effective tool to assess illicit drug use in a community, complementing currently employed methods such as surveys. One of the main issues in determining drug use in a community through wastewater analysis is the drug/metabolite concentration fluctuations due to weather conditions and changes in the population. Creatinine is a product of the muscle metabolism, produced at a relatively constant rate throughout the day. This unique biological material is commonly employed in clinical and forensic toxicology as normalization factor for urine drug concentrations. Although several studies have investigated its utility as a normalization factor in wastewater analysis, the reported results have been contradictory. Further, the analytical methods employed for creatinine determination in wastewater only monitored one transition by liquid chromatography tandem mass spectrometry (LC-MS/MS) and/or utilized complex solid phase extractions.

Objective: The goal of the present study was to develop an analytical method for the determination of creatinine in wastewater samples, and to apply this method to 48 authentic samples collected from 6 wastewater plants in New York City at 8 different time points throughout one year.

Method: Two hundred µL of wastewater were diluted with 200 µL of a solution of creatinine-d_3 at 0.1 µg/mL, and these samples were filtered by Thomson eXtreme/FV® PES 0.2 µm vial filters. Twenty µL of the filtrate were directly injected into the LC-MS/MS (LCMS-8050, Shimadzu) system with electrospray (ESI) in positive mode. The chromatographic separation was performed on a Luna C8 2x150mm, 3 µm (Phenomenex), employing a gradient of 0.1% formic acid in water (A) and in acetonitrile (B). The gradient increased from 2% to 15% B in 1.50 min and then to 95 % where it was held from 3.50 to 4 min. Then, it decreased to 2% from 4 to 4.5 min and was held until 6 min. Two multiple reaction monitoring (MRM) transitions were monitored for creatinine (114>44.05, 114>86.05) and for creatinine-d_3 (117>47, 117>89.1). The analytical method was validated evaluating linearity (n=4), intra- and inter-day precision (n=12), accuracy (n=12), extraction efficiency (n=3), matrix effect (n=48), 96 h autosampler stability (n=3), and interferences. Precision, accuracy, extraction efficiency, and 96 h autosampler stability were evaluated at two concentrations, low QC (0.03 µg/mL) and high QC (3 µg/mL).

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Results: The method was linear from 0.01 to 10 µg/mL (n=4, r^2=0.99979 ± 0.00009). The intra-day precision was 103.5% at low QC and 97.4% at high QC. Inter-day precision was 107.2% at low QC and 104% at high QC. The accuracy ranged from 96.5% at high QC to 102.8% at low QC. Due to the difficulty of collecting authentic wastewater samples from different sources negative for creatinine, we evaluated the matrix effect using creatinine-d_3. Ion suppression was observed (-77.8%, CV=41.8%, n=48). Extraction efficiency was >95.8% at low and high QC; therefore, no creatinine loss was observed due to filtration. Creatinine samples were stable in the filtration vials for at least 96 h stored at 4°C. No interferences from nicotine, cotinine, amphetamines, cocaine, and opioids, were detected. The method was applied to 48 authentic samples collected from 6 wastewater plants in Manhattan, The Bronx, Queens and Brooklyn, at 8 time points (before and after Memorial day, 4th of July, Labor day, and New Year's day). All samples tested positive for creatinine, with concentrations from 0.22 to 2.68 µg/mL, median 1.14 µg/mL. In each wastewater plant, the variation of creatinine concentration was less than 33.8% throughout one year, except in one wastewater plant in Queens (72.8%).

Conclusion/Discussion: A fast and simple method was developed to determine creatinine in wastewater samples with high sensitivity (LOQ 0.01 µg/mL) and specificity (2 MRM transitions per analyte). The developed method could be applied to urine samples (dilution cut-off 20 mg/dL) as well. Creatinine was detected in all authentic wastewater samples with concentrations from 0.22 to 2.68 µg/mL, and it should be further investigated as a normalization factor in wastewater analysis.

Keywords: creatinine, filtration, LC-MS/MS

P13

Cannabinoid Stability in Antemortem and Postmortem Blood

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Background/Introduction: In toxicological testing, drug stability is important. If a drug is degrading before it is tested, interpretation may be affected. Δ-9-tetrahydrocannabinol (THC) decreases in blood over time, but less information is available about the other cannabinoids. In this six-month study, the stability of THC, 11-hydroxy-THC (Hydroxy-THC), 11-nor-9-carboxy-THC (Carboxy-THC), Cannabinol (CBN), and Cannabidiol (CBD) in antemortem and postmortem blood was evaluated at refrigerated (4°C) and frozen (-4°C) storage conditions.

Objective: The objective of this study was to determine the stability of THC, Hydroxy-THC, Carboxy-THC, CBN and CBD in antemortem (AM) and postmortem (PM) bloods in two different storage conditions for six months.

Method: THC, Hydroxy-THC, Carboxy-THC, CBN and CBD were spiked into AM and PM bloods presumptively negative by cannabinoid immunoassay. Pooled blood containing 2% sodium fluoride and potassium oxalate was spiked at target concentrations of 20 ng/mL and 50 ng/mL for THC, Hydroxy-THC, CBN and CBD, and 100 ng/mL and 250 ng/mL for Carboxy-THC for both blood matrices. All aliquots of spiked blood were stored in non-silanized glass vials, mimicking stored casework. Single use aliquots eliminated freeze/thaw cycles. A set of each was refrigerated; another was frozen. Aliquots remained in storage until analysis. Samples were analyzed over a 6-month period, first weekly, then monthly after 8 weeks. The same lot of standards was used for the duration of the study. A Tecan Freedom EVO 200 was used to pipet samples, standards, and reagents. On a 96-well SLE+ plate, 375 µL of a mixture of 150 µL of blood sample, 275 µL of 0.1% formic acid and 25 µL of deuterated internal standard (d3) for all five cannabinoids was added. Vacuum was applied, then drugs were eluted with 1.8 mL of 70:30 hexane:ethyl acetate. Samples were dried with heated air and reconstituted in mobile phase. Separation occured on a Waters Aquity UPLC with HSS T3 column using aqueous and organic phases of 100% water and acetonitrile, each with 0.1% formic acid. The LC method consisted of a 5-minute gradient, eluting all drugs with baseline separation. A Waters XeVo-TQS collected MRM data in ESI+ mode with two ion transitions per compound in the method. The quantitative range is 5-500 ng/mL for Carboxy-THC and 1-100 ng/mL for the other cannabinoids on a quadratic curve, with the administrative LOD set at the LOQ. This method was previously validated with SWGTOX guidelines. Samples were analyzed in triplicate and concentrations averaged. Data was evaluated as percent of original concentration remaining over time. A 20% decrease from original concentration was considered significant based on lab reporting criteria.

Results: Table 1 shows the percentage of each drug remaining after six months. Over the study period 12 aliquots were analyzed, and the mean concentration of each triplicate was used to determine percent remaining. Samples were determined to be stable if more than 80% of the original concentration remained. Most samples did not meet this criterion.

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Table 1. Stability of Cannabinoids in refrigeration/frozen by percent (%) remaining. n = 12

	AM [low]	AM [high]	PM [low]	PM [high]
THC	52.6/23.1	51.7/2.0	8.6/81.5	6.4/56.8
Hydroxy-THC	80.8/43.8	73.2/7.0	21.2/81.4	10.6/66.8
Carboxy-THC	101.9/94.1	89.5/54.0	44.0/94.5	16.5/72.3
CBN	92.0/35.5	80.8/4.1	32.0/80.2	19.8/63.6
CBD	62.7/33.0	61.1/2.7	13.4/75.9	6.8/61.4

Conclusion/Discussion: This stability study expanded on previous studies where often only THC and Carboxy-THC are examined, and looked at other cannabinoids found in human performance and postmortem toxicology. To focus on condition effects, single use aliquots were prepared. Results showed that antemortem bloods are best kept refrigerated rather than frozen, while postmortem bloods show better stability when kept frozen. In comparing low and high concentrations, the low concentration samples tended to have better stability. As with THC, blood samples containing any cannabinoids should be analyzed as soon as possible to attain accurate quantitations, even in optimal storage conditions.

Keywords: Cannabinoids, Stability, Blood

P14

Identification of Novel Synthetic Benzodiazepines by GC-MS

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Background/Introduction: With the rise of novel psychoactive substances, there is also an increase in abuse of novel synthetic benzodiazepines. However, detection of these benzodiazepines can be problematic due to lack of sensitivity in immunoassays and routine screens by gas chromatography-mass spectrometry (GC-MS). Numerous screening methods by liquid chromatography have been developed for synthetic benzodiazepines but detection by GC-MS has yet to be fully evaluated. Novel synthetic benzodiazepines have been reported in several fatalities, both alone and in combination with other drugs of abuse.

Objective: The purpose of this study was to develop a targeted GC-MS screening method for clonazolam, delorazepam, diclazepam, etizolam, flubromazepam, flubromazolam, and pyrazolam in blood. Studies were also performed to compare the optimized method to a routine, full-scan screening method. Further, the techniques were investigated on two instruments with different GC columns.

Method: Solid-phase extraction (SPE) was performed on Clin II SPE columns. The acidic/neutral drugs were eluted with ethyl acetate and the basic drugs were eluted with a dichloromethane/isopropyl alcohol/ammonium hydroxide mixture. During investigation of the SPE techniques, the acidic/neutral and basic fractions were collected separately in order to identify where these synthetic drugs were eluting. GC-MS analysis was performed and compared on two GC-MS with different columns: DB-5MS (30 m x 0.25 mm x 0.10 µm) column and a DB-5 (30 m x 0.25 mm x 0.25 µm) column. The optimized method had an initial oven temperature set at 160°C which was held for 2.0 min and then ramped to 280°C at a rate of 30°C/min with a hold time of 0 min. The oven temperature was ramped to a final temperature of 310°C at a rate of 6°C/min and held for 2.3 min. The sample was injected in pulsed splitless mode. Data was acquired using scan/single ion monitoring (SIM) mode in the optimized screen. The routine screening method had the initial oven temperature set at 160°C for 0.5 min and ramped to 290°C at a rate of 30°C/min and held for 9.167 min. Samples were injected in split mode with a 10:1 split ratio. The routine screen acquired data in scan mode. Extraction recovery was calculated using post-extraction fortification.

Results: The SPE procedure was optimized by collecting both acidic/neutral and basic fractions for benzodiazepines. Diclazepam and flubromazepam were identified in both the acidic/neutral and alkaline elution fractions so routine drug screens collecting only the alkaline fraction may exhibit higher limits of detection due to lower analyte recovery. When both elution fractions were combined, the extraction efficiency was 43.5 – 80.0%. Limits of detection were 50 to 100 ng/mL with the targeted SIM method. The instrument with the thinner stationary phase film (DB-5MS) exhibited lower noise and ultimately offered better sensitivity. Further, increased resolving power was observed with the DB-5MS column as diclazepam and flubromazepam were separated, while these analytes co-eluted on the DB-5 column. The optimized acquisition method, which utilized both scan mode and SIM, was better for identification of these synthetic benzodiazepines due to increased sensitivity compared to the full-scan routine screen. A post-mortem case sample containing etizolam (reported at 62 ng/mL) was analyzed with the optimized SIM method and the routine, full-scan method. Etizolam was detected with the SIM method but was not identified in the full-scan method, which demonstrates that forensic laboratories may miss these synthetic benzodiazepines if only using a routine, full-scan method.

Conclusion/Discussion: If forensic laboratories only rely on immunoassays and routine alkaline screens for identification, then they could be missing these benzodiazepines, especially if they are only collecting the basic fraction from a SPE or utilizing a full-scan GC-MS method. Despite optimization, the targeted SIM method was only sensitive down to 50 ng/mL in blood. Concentrations reported in casework are much lower than this, demonstrating the necessity for a highly sensitive instrument for proper identification of these benzodiazepines.

Keywords: Benzodiazepines, GC-MS, Novel Psychoactive Substances

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P15

Detection of 46 Hallucinogenic-Stimulant Drugs in Urine by Liquid Chromatography Tandem Mass Spectrometry

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Background/Introduction: The Special Forensic Toxicology Drug Testing Laboratory (SFTDTL) was created by the military to help detect new and emerging drugs as part of the Department of Defense (DoD) New and Emerging Drugs Surveillance Program. Our mission is to be at the forefront of new drug trends to help prevent drug use in military personnel. Our laboratory created a new hallucinogenic-stimulant method using DEA Threat Reports, drug forums, and other academic resources, such as National Drug Early Warning System (NDEWS) and Community Drug Early Warning System (CDEWS), to help establish our drug panel. We developed a screen method to detect 46 hallucinogenic-stimulant drugs in urine using liquid chromatography tandem mass spectrometry (LC/MS/MS).

Objective: To introduce a fast, efficient method for detecting 46 analytes in urine utilizing LC/MS/MS.

Method: The panel is composed of a variety of different hallucinogens and stimulants primarily consisting of tryptamines, phenethylamines, piperazines or other compounds related to lysergic acid diethylamide (LSD). The method was developed and validated to detect 46 analytes of interest from a sample size of 500 µL of urine utilizing a quick liquid-liquid extraction. Samples were extracted and reconstituted with 100 µL of mobile phase composed of (90:10) (DIH_2O with 0.1% formic acid: 50:50 methanol/acetonitrile with 0.1% formic acid). Separation occurred on a Zorbax Eclipse Plus C18 Rapid Resolution HD analytical column, (50 x 2.1 mm ID, 1.8 µm) and samples were analyzed on an AB SCIEX 4500 QTrap LC/MS/MS system with a total run time of 6.5 minutes. Validation procedures were based on the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines and in-house validation procedures. The validation consisted of: ion suppression/enhancement, extraction efficiency, selectivity, specificity, limit of detection (LOD, 0.25 ng/mL), carryover, and parallel and stability studies. Specificity was conducted using over 150 different drugs. Once validated, this method was implemented as a routine testing procedure.

Results: The method is currently used to screen Surveillance specimens from military forensic toxicology drug testing laboratories (FTDTLs). The impact of our findings helps to dictate the testing procedures for the military FTDTLs and identifies trends that are used to expand the routine military drug testing panel as needed. Over 6,000 Surveillance specimens have been screened using this new method. The most commonly detected analyte was dextromethorphan. However, some of the more uncommonly seen analytes of interest are ketamine, mitragynine, escaline, 2-oxo-3-OH-LSD, and psilocin.

Conclusion/Discussion: This method is reproducible and robust with a small sample size, simple extraction technique and short run time. The new method enables our laboratory to detect drugs not detected by typical laboratory procedures. Based on this method alone there is a positivity rate of 5%, but this is mostly due to dextromethorphan. The military has a less than one percent drug positive rate due to the fact that some cases are excused via adjudication, medical or legal/command. This is why it is essential that we constantly evaluate and update our screening procedures to ensure that we accomplish our mission of helping to deter and prevent a widespread epidemic of new drug trends.

Keywords: Hallucinogens, Stimulants, LC/MS/MS

POSTER

P16

Improved Novel Psychoactive Substances (NPS) Recognition Using the Randox NPSII Immunoassay in Screening Suspected NPS Deaths

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Background/Introduction: In 2013 there was a significant increase in illicitly manufactured fentanyl availability, which has since evolved into numerous designer chemicals, including analogues of fentanyl. The new designer compounds are now designated novel psychoactive substances (NPS), as chemical diversity continues to expand, and have contributed to the continuing rise in opioid related deaths. NPS availability creates significant challenges for clinical and forensic laboratories to keep pace in detecting additional NPS. Supplementing existing screening methodologies with NPS specific immunoassays is one way to increase the scope of testing, when mass spectrometry detection is not available.

Objective: We retrospectively evaluated the Randox Evidence Investigator immunoassay (IA) NPSII biochip for NPS detection in suspected opioid and fentanyl related death investigations.

Method: Medical examiner cases suspicious for NPS use and which had NPS mass spectrometry (MS) confirmation testing at a reference laboratory performed during the course of routine testing were identified for this study. Two types of cases were found to be suspicious for NPS: 1) due to the scene or case findings or 2) screening was presumptive positive for fentanyl but was not confirmed by subsequent MS testing. Blood and/or urine from these identified cases with remnant specimens were evaluated using the Randox NPSII assay (along manufacturer guidelines). The NPSII biochip assay included the following 14 immunoassays: furanyl fentanyl, acetyl fentanyl, carfentanil/remifentanil, sufentanil, ocfentanyl, U-47700, W-18/W-19/W-15, mitragynine, AH-792, and MT-45.

Results: Twenty specimens (15 = blood, 5 = urine) from seventeen unique cases were tested using the NPSII assay. The Randox NPSII screen improved detection for possible NPS with eleven cases. The blood specimens screened positive in eleven of fifteen specimens, while the urine screened positive in three of five specimens that were all confirmed by MS. Blood specimens were confirmed positive for 4-ANPP, acryl fentanyl, carfentanil, cyclopropyl fentanyl, and mitragynine. Urine specimens were confirmed positive for 4-ANPP, acryl fentanyl, and mitragynine. Three cases in which carfentanil was quantitated in blood at less than 0.2 µg/L were negative by the Randox assay. In the three cases confirmed for acryl fentanyl in blood (0.15, 0.46, and 0.75 µg/L), all three screened positive by the Randox assay.

Conclusion/Discussion: While NPS detection was variable by specimen type and primary detection antibody, the Randox assay was able to identify cases positive for NPS that our current screening approach would have missed. These cases would have been sent out to a reference lab for further testing to aid the medical examiner for cause of death determinations. The Randox system is an excellent, cost effective supplement for initial screening of drugs of abuse.

Keywords: Novel psychoactive substance; Randox; fentanyl

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P17

Evaluation of a low cost urine dipstick for determination of THC use

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Background/Introduction: Immunoassays (IA), particularly enzyme immunoassays (EIA) are frequently the first step of traditional Drug of Abuse (DOA) testing. The Forensic Toxicology Drug Testing Laboratory (FTDTL) at Ft. Meade, MD uses EIA for the initial screen of over 592,000 urine specimens for DOA a year. Specimens with screening results above a threshold value are subsequently quantified by Gas Chromatograph / Mass Spectrometry (GC/MS). Specimens that quantify above the cutoff value and have an intact chain of custody are reported out as positive for the appropriate DOA. IA is a robust methodology that today can deliver quantitative and qualitative results, measure molecules of various sizes, and is widely available to consumers in over the counter (OTC) in vitro diagnostics like glucose monitoring and pregnancy tests. The "Assured™ At Home Marijuana Drug Test" is an IA urine dipstick test that costs $1 and purportedly detects THC at levels of 50 ng/mL and greater with 98% accuracy.

Objective: The objectives of this study were to determine the accuracy of the Assured™ THC test, the ease of interpretation, and how well this OTC IA test performed relative to the high throughput, FDA approved, homogeneous EIA utilized by the Ft. Meade FTDTL.

Method: Screening and confirmation controls were analyzed by the Assured™ THC test. Screening controls consisted of certified negative urine spiked with (-)-11-nor-9-carboxy-THC (THC-COOH). Confirmation controls contained (+)-11-nor-9-carboxy-delta9-THC glucuronide (THC-COOH-Gluc). The concentrations of the screening and confirmation controls were 37.5 ng/mL, 50.0 ng/mL, 62.5 ng/mL, and 1000 ng/mL. A serial dilution of a screening stock solution was used to determine if the Assured™ THC test stated cutoff of 50 ng/mL was supported. Eight authentic urine specimens previously certified as positive for THC-COOH in concentrations ranging from 108 ng/mL to 200 ng/mL were also tested using the Assured™ THC test. Certified negative urine served as the negative control. Specificity of the Assured™ THC test was tested using a cocktail synthetic cannabinoid control containing JWH-018-COOH, JWH-073-COOH, MAM-2201-COOH, UR-144-COOH, AB PINACA-COOH, AB CHIMINACA and 5F-PB-22-COOH at a concentration of 50.0 ng/mL for each analyte. The IA used was the Thermo Fisher DRI® Cannanbinoid Assay analyzed on a Beckman-Coulter AU5830 chemistry analyzer. Confirmation values were obtained by GC/MS using the Agilent 5977 MSD and Agilent 7890B GC systems.

Results: The THC-COOH control was detected by the Assured™ THC test in a concentration dependent manner and serial dilution of this control supports the Assured™ stated cutoff of 50 ng/mL for THC-COOH. THC-COOH-Gluc controls were not detected by the Assured™ THC test or the Thermo Fisher DRI® Cannanbinoid Assay even at THC-COOH-Gluc concentrations up to 1000 ng/mL. Although the authentic urine specimens were all well above the stated 50 ng/mL cutoff, the Assured™ THC test failed to exhibit a consistent linear response to increasing THC-COOH concentrations as it had done with the screening control. Both the Assured™ THC test and Thermo Fisher DRI® Cannanbinoid Assay were insensitive to the seven synthetic cannabinoids at 50 ng/mL. All Assured™ THC test kit control lanes gave a clear, easily readable band indicating a functioning assay.

Conclusion/Discussion: The Assured™ THC dipstick test and Thermo Fisher DRI® Cannanbinoid Assay displayed similar sensitivities for THC-COOH and both assays were specific for THC-COOH over THC-COOH-Gluc and select synthetic cannabinoids. The Assured™ THC dipstick test is easy to use and control materials support the stated cutoff of 50 ng/mL. However, the Assured™ THC dipstick gave unpredictable results in authentic urine specimens known to contain THC-COOH well above 50 ng/mL. This inaccuracy is likely due to the varying amounts of THC-COOH-Gluc in authentic urine specimens. The Assured™ THC test's insensitivity to THC-COOH-Gluc, a predominant THC metabolite in urine, render negative results suspect and only positive results useful when used on authentic urine specimens.

Keywords: THC, dipstick test, accuracy

P18

Postmortem Toxicological Consequences of Bariatric Surgery

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Introduction: The occurrences of bariatric surgery have been on the rise and patients often have multiple indications for pre- and post-operative pharmacotherapy. Procedures target the stomach and/or small intestine and affect weight loss through restriction, malabsorption, or a combination of the two. The absorption and/or metabolism of drugs via the gastrointestinal (GI) tract could be altered by different mechanisms. Several cases at the North Carolina Office of the Chief Medical Examiner's Toxicology Laboratory (OCME) have raised questions about the potential impact of these procedures on the disposition of drugs in the body and how that altered disposition may affect cause and manner of death. Overmedication and postmortem redistribution are not enough to explain the phenomena seen in some OCME bariatric surgery-related casework.

Objective: This presentation aims to provide the toxicology community with a better understanding of the different types of bariatric procedures and a description of how those procedures might affect absorption and/or metabolism of specific analytes of interest. A review of the current literature as it relates to several cases will be presented and evaluated.

Results: Cases that were certified natural, accidental, suicidal, and undermined in manner will be presented and discussed. Case examples include a 43-year-old female with a history of Roux-en-Y gastric bypass (RYGB) who suffered a witnessed collapse. Toxicological findings included elevated concentrations of diphenhydramine at 2.4 mg/L in the femoral blood and 22 mg/kg in the liver. Also, a 67-year-old female, who died from vomiting and bacterial gastritis one day after placement of two intragastric weight-loss balloons, had elevated concentrations of duloxetine at 1.4 mg/L in the iliac vein blood and 9.3 mg/kg in the liver. Her medication was strictly controlled by her sister and gastric contents were without intact tablets or residue at autopsy.

Discussion and Conclusions: The pharmacological consequences of bariatric surgery are not well studied, though it is known that certain classes of drugs, such as anticonvulsants and antidepressants, may be less effective following surgery. Restrictive procedures will increase stomach pH thereby decreasing the absorption of weakly acidic drugs while increasing that of weakly basic drugs (such as duloxetine). Malabsorptive procedures that alter the structure of the small intestine modify the bioavailability of drugs by removing or bypassing the portion of the GI tract that contains metabolic enzymes such as CYP3A4/5 and CYP2C9/19, along with protein transporters such as p-glycoprotein. While it is clinically recognized that drug absorption may be reduced post-surgery, the literature is lacking, and clinical recommendations remain sparse. To the authors' knowledge, this is the first review with a focus on the impact of bariatric surgeries on deaths involving toxicological findings. In compiling these case studies, both clinicians and toxicologists will have a greater appreciation of this complex, multidisciplinary concept.

Keywords: Bariatric surgery, postmortem, drug absorption

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P19

Evaluation of New Specimen Validity Markers

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Background/Introduction: An abundance of synthetic urine products are being marketed for the purpose of specimen substitution for pre-employment, random, or scheduled drug screens. Synthetic urine products have increased in popularity because they meet the criteria for specimen validity testing and yield negative drug screen results.

Packaging materials typically include statements indicating their use for novelty or research use, whereas websites advertising the products provide instructions for urine substitution. Online advertisements also relate that these products are specifically formulated to replicate the physiologic pH, specific gravity, and creatinine concentration of normal human urine. Some indicate that uric acid is also included. Synthetic urine products are so abundant that many are sold online on the Amazon and eBay websites.

Objective: In this study, novel markers of specimen validity were evaluated in order to improve the detection of synthetic urine products. Caffeine, cotinine, theobromine, and urobilin were chosen as markers. We postulated that specimens negative for all four markers would have a high likelihood of being a non-authentic specimen.

Method: The concentrations of caffeine, cotinine, theobromine, and urobilin were measured in urine specimens using liquid chromatography tandem mass spectrometry (LC-MS/MS). The LC-MS/MS method was validated using six-point calibration curves, each with a 5.0 ng/mL lowest level of quantitation. Specimens included those submitted for the purposes of for-cause workplace testing (n=100), pre-employment testing (n=200), and clinical pain management testing (n=100). All specimens collected for-cause involved visual observation, whereas pre-employment and pain management specimens involved unobserved collection.

All specimens were subjected to routine validity tests that included temperature, pH, specific gravity, and creatinine as well as immunoassay testing for common drugs of abuse classes (Amphetamines, Barbiturates, Benzodiazepines, Cannabinoids, Cocaine Metabolite, Methadone, and Opiates). Specimens void of all four markers via LC-MS/MS were subjected to additional testing that involved the evaluation of color, odor, urea, bubbles/foam (after shaking), urinalysis/microscopy, and comprehensive gas chromatography-mass spectrometry (GC-MS) analysis using validated laboratory methods.

Results: Three percent (n=6) of pre-employment and one percent (n=1) of pain management specimens were negative for all four novel validity markers and were labelled “suspicious”. None of the specimens collected with visual observation were void of all four markers. Of the seven suspicious specimens, none exhibited foam/bubbles upon shaking, none contained any biologically formed elements upon microscopic examination, and none contained drugs, compounds, lipids, or hormones commonly detected by our comprehensive GC-MS assay. Of the four markers evaluated, theobromine was the most predominate and was detected in 99.5% of the 393 specimens compared to caffeine (93.3%), cotinine (32.8%), and urobilin (95.8%).

Conclusion/Discussion: The simultaneous application of all four markers appeared to be a robust method detecting synthetic products. It was interesting that each of the seven suspicious specimens exhibited a physiologic temperature, pH, specific gravity, and creatinine. In addition, all seven suspicious specimens were negative for the immunoassay drugs of abuse screens.

Toxicology laboratories need additional markers of specimen validity. Markers may include endogenous compounds that occur in the urine from natural metabolic processes, or may include exogenous compounds that individuals are often exposed to (ie caffeine). While simultaneous detection of the four novel markers in this study appeared to be sensitive and specific, the method required pre-analytical sample preparation and expensive instrumentation. Ideally, the industry will generate new assays to be used on automated analyzers in the common chemical or immunoassay reagent formats.

Keywords: Synthetic Urine, Specimen Validity, Specimen Substitution

POSTER

P20

Method Validation for the Simultaneous Quantification of 18 Commonly Encountered Opioids in Whole Blood by LC-MS/MS

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Background/Introduction: In 2017, a total of 1037 cases were submitted by the Rhode Island Center for the Office of State Medical Examiners for analytical testing by the Rhode Island Forensic Toxicology laboratory. These submissions included 323 cases that were deemed to be accidental drug overdose deaths with 64.1% (207) attributed to fentanyl. Confirmation of fentanyl for these cases was performed at NMS Labs. Liquid chromatography/ tandem mass spectrometry (LC-MS/MS) was employed to validate an 18-analyte panel for in-house opioid analysis to enhance the state's surveillance of accidental deaths attributed to opioids by increasing the number of opioids quantified in post-mortem specimens and reduce the time to obtain laboratory results.

Objective: Develop and validate a LC-MS/MS method that allows for a single sample preparation and quantitation of morphine, 6-monoacetylmorphine, morphine-3β-D-glucuronide, morphine-6β-D-glucuronide, codeine, codeine-6β-D-glucuronide, fentanyl, norfentanyl, hydrocodone, dihydrocodeine, hydromorphone, oxycodone, oxymorphone, methadone, tramadol, O-desmethyl tramadol, buprenorphine and norbuprenorphine in whole blood.

Methods: 800 µL of 0.1 M zinc sulfate and ammonium acetate solution was added to a microcentrifuge tube followed by the addition of 200 µL whole blood aliquots of samples, controls, or calibrators and vortexed 5-10 seconds. A 100 µL mixture of complementary deuterated internal standard (IS) was added to prepared blood samples then vortexed for 30 seconds. Samples were centrifuged and the supernatant diluted with 900 µL of 4% phosphoric acid. Pretreated samples were extracted by solid-phase extraction (SPE) and decanted with a 96-well positive pressure manifold. Samples were washed with 500 µL of 2% formic acid and methanol, respectively; dried at high positive pressure for 3 minutes and eluted with methanol/acetonitrile (75:25) containing 5% strong ammonia. Samples were evaporated to dryness and reconstituted with 100 µL of water/acetonitrile/formic acid (97:2:1) for analysis. Analysis was performed by LC-MS/MS using a Waters ACQUITY UPLC H-Class LC system coupled to a Waters ® Xevo TQD. Mobile phases are: 0.1% formic acid in Milli-Q water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B). The optimized flow rate was 0.4 mL/min using gradient elution with initial conditions of 98:2 (Solvent A: Solvent B) increased to 47.2:52.8 over 6 minutes then returned to 98:2 over 0.5 minutes. The system re-equilibrated for 1.5 minutes with an entire cycle time of 8 minutes. Column temperature was maintained at 30°C and the autosampler maintained at 5°C. Injection volume was 10 µL and the needle was purged with methanol/water (50:50) between injections. Three transitions were monitored for each analyte and internal standard (where possible). Validation was performed using SWGTOX and the RI Forensic Toxicology laboratory guidelines.

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Results: All analytes fit a linear 1/x weighted curve with an $R^2 \geq 0.99$. The LOD ranged from 0.25 and 1.25 ng/mL (analyte dependent). LOQ's were 1.25 and 5 ng/mL (analyte dependent). Percent bias and %CV for all analytes was within acceptable range of +/- 20%. Values obtained with this method were compared with previously quantified samples and yielded acceptable results. Matrix effects and ionization effects were noted and controlled for using matrix matched controls and calibrators with deuterated IS. No significant carryover or interference from drugs of abuse were observed. Extracts were stable 5 days and also quantitated at a 1:2 dilution.

Conclusion/Discussion: A method for the simultaneous quantitation of 18 opioid compounds was successfully validated using SPE and LC-MS/MS.

Acknowledgements: This work was supported by the Centers for Disease Control and Prevention (CDC) Number: CDC-RFA-CE16-16-16080201SUPP17 Supplement to CDC-RFA-CE16-1608 – Enhanced State Surveillance of Opioid-Involved Morbidity and Mortality.

Keywords: Opioids, LC-MS/MS, Whole Blood

P21

Detection of fentanyl in cases of forensic relevance using the SEFRIA™ Fentanyl immunoassay

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Background/Introduction: Worldwide, the illicit use of fentanyl, a powerful synthetic opioid, has increased significantly in the past few years, resulting in soaring numbers of fatal casualties. Fast and reliable detection of fentanyl and its analogues would thus complement the standard forensic screening panel.

Objective: In this study, we tested the performance of the Immunalysis SEFRIA™ Fentanyl immunoassay on paired routine forensic post-mortem urine and serum specimens. The screening results were compared. Sample pairs with concordant positive screenings and those with discordant results were quantified with a validated UPLC-MS/MS routine method, and further validated with LCMSn, as needed.

Method: A total of 74 post-mortem urine and serum sample pairs from autopsy cases were analyzed. The screening of the forensic urine specimens was performed with the SEFRIA™ Fentanyl immunoassay on a Beckman Coulter AU480 in the forensic institute in Salzburg, Austria, at a 1.0 ng/mL cut-off. For the forensic serum samples, the testing was performed in a laboratory in Passau, Germany, with a cut-off of 0.2 ng/mL. Confirmation testing, including fentanyl, norfentanyl, acetylfentanyl, butyrylfentanyl, carfentanil, 3-methylfentanyl, ocfentanil, sufentanil and remifentanil, was performed with a validated UPLC-MS/MS method at a limit of detection (LOD) of 0.02 ng/mL. Standardized, undirected screening with library search was performed on a LCMS-IonTrap (Toxtyper™, Bruker, Bremen) on samples with unclear UPLC-MS/MS results. Confirmation was performed in the MVZ Labor Dessau, Germany.

Results: 66 of the 74 paired post-mortem specimens gave concordant results – 63 resulting negative and 3 reporting positive, the latter 3 containing both fentanyl and norfentanyl. Of the 8 discordant result pairs, one was only borderline positive at 1.2 ng/mL in the urine screen, while the other 7 were positive only in the serum screen. The positive urine screen had to be reported as false positive due to an unspecific crossreactivity to trazodone. Three of the 7 positive serum screens were confirmed – only one did contain fentanyl and norfentanyl. In the second positive specimen only sufentanil, and in the third positive specimen fentanyl, norfentanyl and sufentanil were detected. Further investigation of the third positive sample with a standardized, undirected screening on a LCMS-IonTrap (Toxtyper™) verified the presence of sufentanil and, in addition, a remifentanil metabolite was detected. The remaining 4 positive serum screens did not contain fentanyl or any other of the analogues tested. In summary, the SEFRIA™ Fentanyl Immunoassay showed a sensitivity of 100% and a specificity of 94.1% for the serum matrix, with an overall accuray of 94.6% against confirmation.

Conclusion/Discussion: These data demonstrate that the SEFRIA™ Fentanyl immunoassay is a viable option for the screening of native human urine and serum specimens in a forensic setting. Clearly, the screening of untreated post-mortem serum specimens by immunoassay is more challenging than for ante-mortem serum specimens. Minor adjustments of the sample handling, the sample volume of the screening method and the cut-off level will help to reduce the number of false positive screens.

Keywords: SEFRIA™, Fentanyl, UPLC-MS/MS

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Immunoassay-Based Detection of Fentanyl Analogs in Forensic Toxicology

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Background/Introduction: The abuse of fentanyl and its analogs (fentalogs) is a global growing concern. Forensic toxicology laboratories are tasked with the identification of these emerging drugs in biological matrices. The immunoassay is the most widely used screening technique, in which antigens too small to elicit an immune response require bioconjugation techniques used to attach the target to a larger carrier protein prior to antibody production. The nature of the covalent attachment during haptenation can occlude regions of the drug due to steric hindrance and protein folding. As a result, some regions of the drug may not be recognized by the immune system during polyclonal antibody production. When this occurs, the resulting antibody develops poor specificity for this region. When immunoassays are used to identify several compounds within a class (e.g. benzodiazepines, barbiturates), this moderate to low specificity can be exploited.

Objective: Using five commercial enzyme-linked immunosorbent assays (ELISAs), cross-reactivities towards fentanyl and thirteen fentalogs were investigated to determine their suitability for toxicological screening purposes.

Method: Five commercial immunoassay kits were purchased from Randox Laboratories Ltd (Fentanyl ELISA Plate and Carfentanil/Remifentanil ELISA Plate), Neogen (Fentanyl Group Kit and Fentanil Group Kit), and Immunalysis (Fentanyl ELISA Kit). In accordance with the manufacturer recommendations, dose-response curves were generated using six *N*-propionyl-substituted fentalogs (4-ANPP, acetylfentanyl, butyrylfentanyl, furanylfentanyl, isobutyrylfentanyl, valerylfentanyl), one phenethyl-substituted (norfentanyl), two piperidine-substituted ((+)-cis-3-methylfentanyl, carfentanil), and four phenethyl and piperidine substituted fentalogs (alfentanil, norcarfentanil, remifentanil, sufentanil). Cross-reactivities were evaluated at three levels (EC_{50}, 0.5 ng/mL, and 1 ng/mL) for each assay to determine its overall effectiveness for fentalog screening in urine.

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Results: The cross-reactivities were relatively consistent across the three calculation methods. Three of the kits were able to identify fentalogs substituted at the *N*-propionyl position and two were effective with respect to the piperidine and phenethyl groups. However, none of the kits evaluated produced sufficient cross-reactivity to detect all of the fentalogs that were tested.

Conclusion/Discussion: Several commercial assays proved effective for the identification of either *N*-propionyl or piperidine-substituted fentalogs, but none was capable of identifying both. Although this is an inherent disadvantage of the immunoassay approach, it arises from the diverse structural nature of the fentanyl analogs themselves. It would be necessary to use more than one immunoassay kit to screen for emerging fentalogs. This may be cost prohibitive and highlights the challenges associated with novel psychoactive substances (NPS) in general. Alternative mass spectrometry (MS)-based screening methods may be more responsive to the proliferation of NPS moving forward.

Keywords: Fentanyl, Immunoassay, ELISA

P23

Using LC-QTOF-MS in validated screening workflows in forensic toxicology: A qualitative/quantitative method for 93 drugs of abuse in human urine samples

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Background/Introduction: Full-scan based screening methods using LC-QTOF-MS are a valuable tool for forensic analysis due to the possibility of qualitative/quantitative and retrospective data evaluation. In this study, a previously developed LC-QTOF-MS screening workflow was validated for qualitative and quantitative analysis of drugs and drugs of abuse in human urine.

Objective: A basic validation including limits of detection, limits of quantitation, linearity, accuracy, selectivity, and precision was carried out. Furthermore, the methods' limitations regarding its applicability to urine screening in post-mortem toxicology, workplace drug testing, drug-facilitated crime (DFC), and intoxication cases were evaluated. Special focus was given to prove that cut-off values for sobriety and fitness-to-drive testing were met.

Method: Ninety-three substances of forensic relevance were spiked into ten different urine samples at the concentrations 1.0, 5.0, 10, and 50 ng/mL. Samples were precipitated with acetonitrile after addition of internal standards. The dried residues were reconstituted and analyzed on three LC-MS systems (Elute Liquid Chromatography system coupled to a Bruker impact II QTOF). Separation was performed on a C18 column using a 14 min gradient elution. The MS was operated in positive electrospray ionization mode generating a full scan and broadband CID spectra (bbCID) using a collision energy spread (24 – 36 eV). Data evaluation was performed with TASQ software using a database containing mass spectrometric and chromatographic information on 2184 compounds.

Results: Identification and semi-quantification at the lowest concentration (1.0 ng/mL) were achieved for 60 % of the compounds; five compounds (acetaminophen, norclomipramine, piritramide, THC-COOH, and levetiracetam) were not detected, due to matrix effects and/or low ionization yield. LOD was set to the concentration at which a substance was identified in 95% of all analyses conforming with the following identification criteria: retention time ± 0.3 min, signal-to-noise ratio > 3:1 for all ions, [M+nH]n+ and [M+nH+1] n+ detected (MS), at least two qualifier ions with at least one being a true fragment of the molecular ion (bbCID). The precision ranged from 8 % to 30 %.

Conclusion/Discussion: All substances with legal cut-offs according to German regulations (CTU3 criteria for abstinence screening in fitness-to-drive assessment) were found well below the respective concentrations (factor 5). Noteworthy, designer benzodiazepines and fentanyl derivatives were detected with high sensitivity. Typical 'date rape drugs' like flunitrazepam, doxylamine, and diphenhydramine showed LODs sufficient for detection of a recent uptake of the drugs.

Extrapolating the here presented urine analysis results, application to blood and hair samples seems promising and should be tested/validated. Given the high frequency of new psychoactive substances emerging on web-based drug markets and related fatalities, this is of particular interest due to the possibility of retrospective data evaluation.

Keywords: LC-QTOF, Urine drug Screening, qual/quant

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P24

Analysis of Cannabidiol (CBD) and Δ^9-Tetrahydrocannabinol (Δ^9-THC) in CBD Oil/Hemp Oil Products

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Background/Introduction: Hemp products are readily available and are marketed for their health and medicinal benefits. It is widely believed that these products contain the highly sought after compound CBD that has taken the natural products industry by storm. The CBD and Δ^9-THC levels in these products are unknown, and the validity of the amounts in the products label, when given, is not confirmed.

Objective: In order to gain a better understanding of the CBD content, forty-nine hemp products were analyzed by gas chromatography coupled with mass spectrometry (GC-MS) for CBD, Δ^9-THC, Tetrahydrocannabinolic acid (Δ^9-THCA), and cannabidiolic acid (CBDA). Δ^9-THCA and CBDA are the natural precursors of the Δ^9-THC and CBD in the plant material. Decarboxylation to THC and CBD is essential to get the total benefit of the neutral cannabinoids. Therefore, testing for the neutral and acid cannabinoids is important to obtain a complete picture of the chemical profile of the products.

Method: A total of 50 to 100 mg of material was weighed and diluted with hexane to make 10 mg/mL solutions. A volume of 1 mL (straight, equal to 5 or 10 mg of oil) and 0.1 mL (dilute, equal to 0.5 or 1 mg of oil) of the hexane solutions were spiked with d_3-Δ^9-THC and d_3-CBD. The solutions were adjusted to 5 mL with hexane and vortexed. To this, 4 mL of 0.2 N sodium hydroxide was added and mixed. The solution was centrifuged, and the top layer (hexane) was discarded. A volume of 1.5 mL of 1 N HCl was added to the basic layer and mixed, with the pH checked to be between 1 and 2. A volume of 1 mL of hexane was added, and the sample was mixed and centrifuged. The top layer was transferred to a GC vial and evaporated. The sample was derivatized using N,O-Bis(trimethylsilyl)-trifluoroacetamide (BSTFA) to make a TMS (trimethylsilane) derivative, followed by analysis using GC-MS. The GC-MS method was developed and validated. A 10 meter x 0.18 mm DB-1 (0.4 μ film) column was used for the analysis.

Results: The majority of the hemp products were oils, one of the products was hemp butter, one was a concentrated hemp powder capsule, and another was a hemp extract capsule. Most of the products contained less than 0.1% CBD and less than 0.01% Δ^9-THC. Three products contained 0.1% to 1% CBD, and two products contained 0.1% to 0.9% Δ^9-THC. Only four products contained significant amounts of CBD. All of the samples appeared to be decarboxylated since the CBDA and Δ^9-THCA results were less than 0.001%.

Conclusion/Discussion: Out of the forty-nine hemp products analyzed, most of the products contained CBD and Δ^9-THC at very low concentrations; only four products contained significant quantities of CBD. All of the samples appeared to be decarboxylated because the CBDA and Δ^9-THCA results were less than 0.001%.

Keywords: CBD Oil, Hemp Oil, GC-MS

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A Retrospective Study on the Use of Opiates with Fentanyl from 2013-2018

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Background/Introduction: Historically, prescription opioids, particularly hydrocodone, were prevalent in postmortem and human performance casework in Harris County, Texas. Perhaps due to more stringent restrictions of prescription opioids, there has been a surge in the use of heroin and fentanyl.

Objective: A retrospective study was completed to evaluate morphine, 6-acetylmorphine (6-MAM), codeine, hydrocodone, hydromorphone, oxycodone, oxymorphone and fentanyl prevalence in medicolegal and human performance toxicology cases requested from January 1, 2013 and April 1, 2018.

Method: All cases were analyzed as part of the normal testing scheme at the Harris County Institute of Forensic Sciences. Screening was performed by enzyme-linked immunosorbent assay. Opioids and fentanyl were confirmed using separate methods that both employed liquid-liquid extraction and liquid chromatography tandem mass spectrometry (LC/MS/MS) analysis. The limits of quantitation for opioids and fentanyl were 0.010 mg/L and 1 µg/L, respectively. Since heroin metabolizes to 6-MAM within minutes, urine and/or vitreous humor in medicolegal cases were often analyzed in conjunction with whole blood due to the longer detection period of these matrices. In human performance cases, blood and/or urine were tested as required. Opioids- and/or fentanyl-positive cases were identified by searching the laboratory information management system. "Positive" is defined as the presence of an analyte in at least one matrix; to be "heroin-positive," 6-MAM had to be present.

Results: Medicolegal

Over the last five years, the prevalence of non-fentanyl opioids have remained relatively stable, with 312-401 positive cases each year, which was 10-13% of all medicolegal cases. Hydrocodone was the most prevalent opioid identified in 2013-2015 and the second-most prevalent opioid in 2016 and 2017, behind morphine. Morphine and codeine demonstrated a steady increase in the number of positive cases, but hydrocodone did not have a reciprocal reduction, suggesting some level of poly-opioid use. There also has been a steady increase of fentanyl-positive cases, alone or in combination with other opioids, from 22 in 2013 to 53 in 2017. Fentanyl was rarely found in combination with another opioid from 2013-2016, constituting less than 5% of all opioid-positive cases, until 2017, when it nearly doubled. Hydrocodone, oxycodone and their metabolites were consistently the opioids that were found in combination with fentanyl from 2013-2016, making up 67-86% of the cases. In 2017, fentanyl and hydrocodone/oxycodone/metabolite combinations decreased drastically to 7% and were replaced by fentanyl and heroin. In 2018, the high prevalence of heroin and fentanyl continues.

Human Performance

Approximately 3700 human performance cases received drug testing during this five-year period; of these, 22% contained opiates and less than 1% contained fentanyl. Hydrocodone and morphine accounted for 51-72% and 12-25% of the opioid-positive cases, respectively. Unlike the medicolegal cases, no clear trend of increasing morphine use was observed. In 2013, there were no cases of fentanyl combined with opioids. From 2014-2017, the number of fentanyl combined with opioids ranged from 1-4 cases with 2 cases already reported in 2018; combination cases constitute less than 3% of all opioid-positive cases. Hydrocodone and morphine were the two most predominant opioids combined with fentanyl.

Conclusion/Discussion: The combination of fentanyl and opioids in postmortem cases is not a new trend for other areas in the United States, but Harris County, Texas, was more resistant to the shift in which opioids are used. Hydrocodone was one of the most prevalent opioids seen in Harris County from 2013-2015 and was the most common opioid combined with fentanyl. Since 2017, we have experienced a surge in fentanyl and fentanyl combined with heroin, perhaps due to decreased accessibility to hydrocodone. Additional data, including drug seizures, are necessary to establish whether this trend will continue.

Keywords: Fentanyl, Opioids, Heroin

P26

Opioid Quantitation using DPX Tips and Semi-Automated Integra Pipetting System on an Agilent Triple Quadrupole Mass Spectrometer

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Background/Introduction: According to the Centers for Disease Control and Prevention, overdose deaths involving prescription opioids were five times higher in 2016 than 1999. The age-adjusted rate of drug overdose deaths involving synthetic opioids other than methadone (e.g. fentanyl, tramadol) doubled between 2015 and 2016, from 3.1 to 6.2 per 100,000. The increase in opioid related incidences in DUI/DUID and overdose related deaths has resulted in an increase in cases needing confirmatory testing. It is desirable to have a method that is efficient, robust, and capable of detecting and quantitating a large number of opioid analytes.

Objective: To develop a semi-automated dispersive pipette extraction (DPX) method for the analysis of opioids in whole blood using an LC-QQQ in an effort to replace an existing Solid Phase Extraction (SPE) and Gas Chromatography/Mass Spectrometry (GC/MS) analysis, and expand the laboratory's detection and quantitation capabilities to include oxymorphone, hydromorphone, and tapentadol.

Method: Drug standards were purchased from Cerilliant Corporation and Lipomed. DPX WAX tips were purchased from DPX Labs, LLC (Columbia, SC). DPX WAX tips use a reverse phase and anionic exchange resin with styrene dibenzene to remove non-polar substances and phospholipids that are sources of ion suppression in whole blood. The extraction procedure was performed using an Integra semi-automated pipetting unit with DPX WAX tips. Instrumental analysis was performed using an Agilent 1290 LC coupled to a 6430 QQQ equipped with a Restek Raptor™ Biphenyl 2.1 x 100 mm, 2.7 µm column. The following analytes were included: codeine, morphine, 6-monoacetylmorphine (6-MAM), hydrocodone, hydromorphone, oxycodone, oxymorphone, fentanyl, tapentadol, tramadol, methadone, and meperidine. Mobile phases consisted of 5 mM Ammonium Acetate with 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). Injections of 10 uL were introduced into a gradient elution from 98% A to 98% B over 9 minutes. SWGTOX method development and validation guidelines were followed and included accuracy and precision, limits of detection, linearity, calibration model, matrix and analyte interference, carryover, matrix effects/ion suppression, dilution integrity, and stability.

Results: Between run and within run accuracy was acceptable and within +/- 20% of the intended target concentration. The lower and upper limits of quantitation were determined to be 0.5 and 100 ng/mL respectively for oxymorphone, fentanyl, hydromorphone, and 6-MAM, and 5 and 1,000 ng/mL for morphine, meperidine, tapentadol, codeine, oxycodone, hydrocodone, tramadol, and methadone. Linearity was assessed and all targets were best fit with a quadratic fitting algorithm with a weighting of 1/x. No carryover was detected following concentrations of 100 ng/mL for oxymorphone, fentanyl, hydromorphone, and 6-MAM, and 1,000 ng/mL for morphine, meperidine, tapentadol, codeine, oxycodone, hydrocodone, tramadol, and methadone.

Conclusion/Discussion: A semi-automated sample preparation and quantitation method for the analysis of opioids in whole blood was successfully developed. This allows for a more comprehensive scope of opioid analytes within a single method, a wider linearity range, and combines separate methodologies commonly employed for the analysis of opioids in DUI/DUID and post mortem analyses. A high percentage of our monthly caseload currently produces presumptive positives within the aforementioned analytes. This methodology will decrease extraction/analysis time by more than 50% and in combination with LC-QQQ technology, allows for an overall more efficient analysis than is currently employed. The work presented here will benefit other Forensic Laboratories in the analysis of opioids using semi-automated technology coupled with LC/QQQ analysis.

Keywords: Semi-automation, dispersive pipette extraction, Opioid

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The Most Prevalent Drugs Found in Child Endangerment Cases in Alabama

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Background/Introduction:

Child endangerment is when a person engages in conduct that places a child in imminent danger of death, bodily injury, or physical or mental impairment. This is a Class A misdemeanor in Alabama. However, if a responsible person commits the crime of chemical endangerment of exposing a child to an environment in which he or she does any of the following, it is a felony:

(1) Knowingly, recklessly, or intentionally causes or permits a child to be exposed to, to ingest or inhale, or to have contact with a controlled substance, chemical substance, or drug paraphernalia.

(2) Violates subdivision (1) and a child suffers serious physical injury by exposure to, ingestion of, inhalation of, or contact with a controlled substance, chemical substance, or drug paraphernalia.

(3) Violates subdivision (1) and the exposure, ingestion, inhalation, or contact results in the death of the child.

Objective: We determined the most prevalent drugs found in child endangerment cases over a 6.5 year period and highlighted two representative cases. In addition, we explored the demographics and constructed maps based on prevalence data.

Method: Data for this project was mined from the ADFS LIMS. From 2012 to mid-2018, there were 323 cases submitted as child endangerment cases. These cases included samples primarily from mothers and newborns that typically included blood, urine, cord blood and meconium. Toxicological examinations included analyses for ethanol and related volatiles by HS/GC-FID, immunoassay drug screening using a Randox Evidence Analyzer, and drug confirmation by liquid-liquid and solid-phase extractions followed by GC/MS and/or LC/MS/MS. When warranted, quantitative analyses were conducted. Prevalence data was used to construct geo-maps which is the visual representation of collected data overlaid on a map to show the results of said data.

Results: 204 of 323 (63%) of cases submitted were positive for drugs. Surprisingly, only four cases were positive for ethanol. 139 of 323 (49%) cases had multiple drugs detected. From 2014 to 2015 there was a 45% increase in case submissions and an additional 26% increase from 2016 to 2017. Geo-mapping indicated 3 of 67 counties submitted 63% of the positive cases and 65% of the total number of cases submitted. Methamphetamine and amphetamine were the most prevalent drugs found in 47% of total cases submitted. The median (range) for methamphetamine and amphetamine concentrations were 171 ng/mL (< 10 ng/mL to 1200 ng/mL) and 40 ng/mL (10 ng/mL to 210 ng/mL), respectively.

Conclusion/Discussion: Since the addition of the chemical endangerment clause in the Alabama child endangerment law, many counties in the state have greatly increased their efforts to combat their growing drug problems. From 2012 to mid-2018, three counties led the charge for using the chemical endangerment clause to prosecute drug users. The clause was originally intended to combat the drug problem by prosecuting illicit drug manufacturing (e.g. meth labs). Initiatives by these local district attorneys and drug courts have now shifted their efforts towards pregnant mothers and mothers of newborns. The statistics presented here show that these counties have a significant problem of mothers exposing their children *in utero* to illegal drugs. There is some debate regarding the effectiveness of prosecuting mothers that expose their unborn children to drugs as a deterrent to drug use. Also, of concern is how many individuals are being charged based solely on hospital testing, where a confirmation analysis may not be conducted. In all suspected cases law enforcement should be encouraged to submit samples to crime laboratories for confirmation testing and discouraged from relying solely on preliminary hospital screens. It is important for forensic toxicology laboratories to communicate with law enforcement to ensure that proper sample collection and testing occurs.

Keywords: Child endangerment, chemical endangerment, methamphetamine

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Riding the Dragon: Optimization of Methadone Recovery in an E-cigarette Aerosol with the Addition of Caffeine

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Background/Introduction:

Methadone, a schedule II opioid is commonly used for the treatment of opiate dependency and pain management. As an opiate analgesic that acts as a ì-opioid agonist, methadone versus morphine studies show a much longer half-life in methadone administration. Electronic cigarettes (e-cigs) have been adopted to inhale drugs other than nicotine (DOTNs), and online forums have discussed the potential for vaping opioids, including methadone. Caffeine is frequently used as a cutting agent for opioids to improve aerosolization when smoking, and users have suggested that adding caffeine to e-liquids will improve the aerosolization of opioids in e-cigarette aerosol.

Objective: The purpose of this study is to determine whether the dose of methadone aerosolized by a KangerTech AeroTank is improved by the addition of caffeine to an e-liquid.

Method: An e-liquid was formulated containing 10 mg/mL of methadone and 5 mg/mL of caffeine (2:1) in a 50:50 propylene glycol (PG):vegetable glycerin (VG) solution. The e-liquid was aerosolized into a water trap using a KangerTech AeroTank for 4 seconds. The e-cigarette was operated at 4.3 V (n=5). Aliquots from the trap were extracted with 1-chlorobutane and ammonium hydroxide and analysis was performed on an Agilent GC/MS 6890N/5973 Mass Selective Detector instrument with an Agilent HP-5MS column (0.25mm x 30 m x 250 µm). Each sample was analyzed in split mode 20:1. The initial temperature of the oven was 225°C with a ramp of 15°C /min to reach a final temperature of 285°C and a total run time of 4 minutes. The MSD was run in SIM mode with ions 72, 223, 294, and 309 m/z monitored for methadone; 78, 226, 303, and 318 m/z monitored for methadone-d9, and finally 67, 109, and 194 m/z monitored for caffeine. A calibration curve was prepared from 100-5000 ng/mL.

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Results: The aerosol was confirmed to contain methadone, caffeine, PG, and VG by GC/MS. The average theoretical dose concentration of aerosolized methadone was 257 µg (239-278 µg) (6 % CV), and the experimental dose determined by GC/MS was 177 µg (159-199 µg) (9 % CV). Percent recovery for the aerosolized methadone with caffeine was 69% (64-77% with 8% CV).

Conclusion/Discussion: A previous study with an e-liquid containing 10 mg/mL methadone demonstrated, on average, 8% recovery of methadone in each puff (6-13% with 39% CV) at 4.3 V. Comparatively, the addition of caffeine to the methadone e-liquid resulted in 69% recovery (64-77% with 8% CV). Methadone is capable of being aerosolized in e-cigarettes. However, it is an inefficient aerosolization, conceivably due to its 390°C boiling point being outside of the average operating temperature of the e-cig devices of 200°C. The addition of caffeine potentially creates a eutectic mixture that lowers the boiling point of the drug mixture and improves its aerosolization and bioavailability. Since this technique is advised in several drug forums, it is important for the community to know what to analyze and how to interpret cases which involve high boiling point drugs like opioids.

Keywords: Methadone, Caffeine, Electronic Cigarettes

P29

Chloroethane Stability and Distribution in Four Fatal Cases using HSGC-FID and HSGC-MS

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Background/Introduction: Chloroethane is a colorless gas and is commercially available in canisters marketed as cleaning solvents. Recreational misuse of chloroethane by inhalation of the pressurized contents of these canisters, causes marked central nervous system depression. Deaths resulting from misuse of chloroethane are not commonplace and as such, there is limited information in the literature on the stability or distribution of chloroethane in different postmortem specimens. This is of particular importance when selecting the most appropriate specimen for analysis in challenging cases where femoral blood may not be available. Over a period of 13 years (2005 to 2018), twelve fatal cases attributed in part or wholly to chloroethane were identified within the NYC Office of Chief Medical Examiner. Four of the twelve cases had specimens retained following refrigerated and/or freezer storage to allow further investigation.

Objective: To re-test specimens from four cases received between 2015 and 2018 to determine the stability of chloroethane following storage under different conditions. In addition, to carry out an assessment of the distribution of chloroethane in different specimen types to determine which matrices are preferable to test for chloroethane and what alternative matrices can be used if those preferred are not available.

Method: Femoral blood and vitreous humor specimens for all four cases were retested after a storage period ranging from 2 months to 28 months in a refrigerator or freezer. Specimen preparation was minimal and involved dilution with internal standard (n-propanol in deionized water). Analysis was conducted using an Agilent Technologies 7697A Headspace Autosampler and 7890B GC System with dual flame-ionization detectors and Restek RTX-BAC1 and RTX-BAC2 columns. All samples were also re-tested using an Agilent Technologies HSGC/5977A MSD equipped with a RTX-BAC1 column. In addition, specimens of heart blood, liver blood, bile, urine, gastric contents, and brain were available for some but not all cases and were also tested to evaluate chloroethane distribution.

Results: Chloroethane was still detected in femoral and vitreous humor for all four cases following short and long-term storage. However, in femoral blood, decreases of as much as 96% (28 months in storage) and 81% (2 months) were observed. For the same cases, the corresponding loss was less marked in vitreous specimens, at 65% and 45% respectively. Choroethane was detected in heart blood, liver blood and brain in all cases where they were available, in agreement with the corresponding femoral blood specimens. Chloroethane was detected in bile in two cases but not detected in a third. Importantly, in one of the positive bile specimens, ethanol was also present and as highlighted in previous publications, ethanol co-elutes with chloroethane on a BAC-1 column; however, separation was observed on the BAC-2 column and analytes were further distinguished using the HSGC-MS. Chloroethane was detected in some but not all urine specimens and gastric contents.

Conclusion/Discussion: Despite its volatility, chloroethane was detectable in blood and brain following more than two years of storage. Significant loss was observed for femoral blood compared with vitreous humor. This could be a reflection of the number of multiple cycles of freezing and thawing, room temperature equilibrations, and the opening of sample containers for additional testing. A dedicated "volatiles" specimen was not available for the four cases investigated and would be recommended. Both liver blood and brain could prove suitable as alternative specimens for cases where femoral or heart blood is not readily available.

Keywords: chloroethane, inhalant, HSGC-MS

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Drug Facilitated Crimes: Expanded Workflow and Trends Observed in Miami-Dade County, Florida

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Background/Introduction: In Miami-Dade County, the number of suspected drug facilitated crime (DFC) cases in which toxicological analysis was requested has increased annually since 2013. The University of Miami Toxicology Laboratory (UMTL) is responsible for processing these cases and has recently implemented a specific workflow for DFC cases which differs from the laboratory's workflow for driving under the influence investigations. This new workflow evaluates the types of specimens submitted for a case and the approximate time window between the incident and sample collection to effectively utilize laboratory resources. This workflow includes additional screening and confirmatory analytical methods to encompass a broad range of drugs and metabolites and to make an effort to meet the "Recommended Minimum Performance Limits" by the SOFT DFC Committee. With the increase in DFC cases, the UMTL was interested in evaluating trends to determine if further modification of the workflow is necessary.

Objective: The purpose of this study was to examine the submission and drug usage trends and to determine the most commonly reported drugs and metabolites in suspected DFC cases that were submitted to the UMTL for toxicological analysis since 2013.

Method: All of the completed DFC cases that were submitted to the UMTL between January 2013 and April 2018 were reviewed. Case submission rates were recorded. Also, the presence and concentration of alcohol, if applicable, and drugs and metabolites that were reported for each case were recorded. This data was analyzed to determine drug trends, the number of drugs and metabolites reported per case, and commonly reported drugs and metabolites.

Results: The results of this study show the number of suspected DFC cases submitted to the UMTL has steadily increased in recent years from 59 cases in 2013 to 102 cases in 2017, with 42 cases submitted as of April 2018. DFC cases comprised approximately 15% of overall case submissions in 2013, but by 2017, this increased to 33%. DFC cases submitted to the UMTL for toxicological analysis are commonly suspected drug facilitated sexual assault (DFSA) cases. Over the past 5 years, ~ 50% of all DFC cases reported the presence of drugs without ethanol. In 23% of the DFC cases, ethanol and drugs were both reported, in 10% of the DFC cases the presence of ethanol without drugs was reported, and in 19% of the DFC cases no ethanol or drugs were reported. In 2013, 12% of the cases had 6 or more drugs and/or metabolites reported; this has increased to 21% in 2018.

Historically, the compounds associated with DFSA include GHB, flunitrazepam, and ketamine; however, these compounds have not been detected in any DFC case submitted to the UMTL between 2013 and 2018. In the more than five years of DFC casework that was reviewed for this study, the most commonly reported compounds, not including ethanol, are (±)-11-nor-9-carboxy-Δ^9-THC (43%), cocaine (18%), cocaine metabolites (19%), and alprazolam and/or its metabolite (14%). Synthetic cathinones have also been detected in DFC cases. In 2013, methylone was reported in 11% of DFC cases. Throughout the years other synthetic cathinones reported include ethylone, **α-pyrrolidinovalerophenone** (α-PVP), and dibutylone. Beginning in 2017, n-ethylpentylone has been detected in 5% DFC cases.

Conclusion/Discussion: The number of DFC cases submitted for testing has increased annually and comprises a greater percentage of current casework. Consequently, the UMTL determined it was necessary to optimize the workflow of these cases to ensure that laboratory resources were being used efficiently and to increase the analytical sensitivity for DFC drugs. The increased number of drugs reported in DFC cases may be due to increased drug usage, additional testing, lower limits of detections, or a combination of these factors. Based on the percentage increase of cases positive for six or more drugs and/or metabolites from 2013 to 2018 and the identification of novel psychoactive substances, specifically synthetic cathinones, the DFC-specific workflow enabled the detection of a broad range of drugs and metabolites.

Keywords: drug facilitated crimes, trends, workflow

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Quantitative Measurement of PB-22 and 5F-PB-22 in Oral Fluid

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Background/Introduction: Over the last ten years, new designer drugs have gained significant popularity among drug users. When the drug enforcement authorities find the presence of new designer drugs in the market, they add the new drugs to the legislation. As new designer drugs are added to the legislation, more designer drugs are being introduced to the market. Synthetic cannabinoids are one of the classes of new designer drugs. PB-22 and 5F-PB-22 are two new synthetic cannabinoids that have been added to Schedule I controlled substances list by the United States Drug Enforcement Administration. To test collected oral fluid samples for PB-22 and 5F-PB-22, we developed a bioanalytical method that initially purifies the sample with solid phase extraction and then quantitatively identifies the drugs with Ultra High Performance Liquid Chromatography—Tandem Mass Spectrometry (LC-MS-MS). The validation data showed that the method is an accurate, precise, robust and efficient method suited for high throughput toxicological confirmation applications.

Objective: To develop and validate LC-MS-MS methods that can accurately quantify PB-22 and 5F-PB-22 in oral fluid samples.

Method: Oral fluid samples were collected with Quantisal™ (Immunalysis) collection devices, which consist of a cotton pad and an extraction buffer. According to the specifications of the manufacturer, under the routine collection procedure, the cellulose pad absorbs 1 mL (±10%) of the oral fluid. There are 3 mL of extraction buffer in the tube. Therefore, the dilution factor of neat oral fluid in samples is 1:4. Because the original oral fluid was diluted with extraction buffer during the collection, detected drug concentrations were multiplied by four. 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 Agilent PCX 96 Well Plate. Only 400 µL of the collected sample was used for the SPE. Chromatographic separation was performed on an Acquity UHPLC I-Class system (Waters) equipped with Zorbax Eclipse C8 RRHD analytical column (Agilent Technologies). Electrospray ionization mass spectrometry was performed on a TQ-S instrument (Waters). Analysis was performed in positive ionization (ESI+) and multiple reaction monitoring (MRM) mode. Two transitions, quantifier and qualifier, were used to identify the target analytes. MRM transitions for the parent drugs were as follows: 359.2>214.1 m/z and 359.2>144.0 m/z for PB-22 and 377.2 >232.1 m/z and 377.2 >144.0 m/z for 5F-PB-22. Authentic samples were tested with the current method to evaluate the applicability of the method.

Results: The developed methods were validated according to Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines. The linear dynamic range for PB-22 and 5F-PB-22 was 0.1-10.0 ng/mL. The acceptance criterion for the accuracy and precision was that % relative error and % CV should be ≤ 20% for QC samples. The results of the accuracy and the precision values were within the acceptance criteria for all the analytes. PB-22 and 5F-PB-22 shows ion enhancement effect in LC-MS-MS analysis. Average recoveries of PB-22 and 5F-PB-22 in SPE were 77.5% and 80.9%, respectively. In addition, selectivity was calculated for the LC-MS-MS method.

Conclusion/Discussion: The validation data indicated that the method was accurate, precise and robust. This method is suited for toxicology confirmation applications in a production setting.

Keywords: PB-22, 5F-PB-22 and Oral Fluid

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Validation of Randox Carfentanil Immunoassay on Tecan Freedom Evo 75

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Background/Introduction: Carfentanil is an analogue of fentanyl, a synthetic opioid commonly used to treat severe pain associated with cancer and other terminal illnesses. Carfentanil, however, is typically used as a tranquilizer for large mammals, such as elephants. Its potency is 10,000 times that of morphine and 100 times that of fentanyl. Carfentanil has recently been implicated in the increase in overdose deaths related to fentanyl analogues. Due to the increased potency of carfentanil, even habitual users with a high tolerance for opioids are susceptible to overdose. Increasing concern regarding carfentanil amongst law enforcement and medical personnel has led to a need for a robust screening method. The Tecan Freedom Evo 75 using the Randox ELISA kit provides a sensitive and quick immunoassay screening option in whole blood for both human performance and post-mortem applications.

Objective: To validate the Randox Carfentanil ELISA kit in 96-well format in whole blood using a semi-automated Tecan Freedom Evo 75 instrument.

Method: Immunoassay kits were purchased from Randox Laboratories containing 96-well plates coated with the polyclonal carfentanil antibody. The procedure used was based on the manufacturer's recommendations. Optical density was measured at 450 nm by UV/VIS spectrophotometry. The cutoff for carfentanil was set to 0.8 ng/mL by the manufacturer. Precision at 50% below the decision point (low – 0.4 ng/mL), at the decision point (cutoff – 0.8 ng/mL), 50% above the decision point (1.5X – 1.2 ng/mL), and 100% above the decision point (high – 1.6 ng/mL) was monitored in triplicate over five days for carfentanil. A total of three previously analyzed samples, as well as eight simulated samples, were evaluated to determine false positive/false negative rates and to assess the assay's ability to reliably detect compounds at concentrations commonly observed in routine casework. Interferences were evaluated with eight ante- and eight post-mortem blood specimens and with 14 commonly encountered drugs. Intra-assay precision, limit of detection, and cross-reactivity have been previously validated by Randox as a component of their in-house validation.

Results: Intra-day precision (%CVs) was 2.8% – 17.9% for carfentanil. Between-day precision (%CV) was 6.79% for the low control, 13.3% for the cutoff, 10.8% for the control at 50% above the cutoff, and 9.26% for the high control. All concentration points had CVs less than 20%.

The 16 previously determined negative ante- and post-mortem cases showed no matrix interference. There were no false positive results. Of the three known carfentanil positive blood samples, only one was presumptively positive at a cutoff of 0.8 ng/mL. The other two cases were elevated, meaning the absorbance was higher than the cutoff but less than the 0.4 ng/mL low positive control (absorbance is inversely proportional to concentration). The two cases that were negative in this experiment were previously sent to a reference lab, and both quantitative results were below the cutoff (0.8 ng/mL) of this assay. Urine and vitreous humor accompanied two of the three blood samples. Those samples were all presumptively positive for carfentanil. The eight simulated samples ranged in concentration from 0.39 ng/mL – 4.0 ng/mL. Of the eight samples, five (0.54, 0.68, 1.8, 2.0, 4.0 ng/mL were presumptively positive for carfentanil. The remaining three samples (0.39, 0.72, and 1.0 ng/mL) were elevated.

Conclusion/Discussion: We demonstrated that the Randox Carfentanil ELISA assay was fit for purpose in multiple specimen types. There were no false positives, but there were two false negatives at the cutoff recommended by the manufacturer (0.8 ng/mL). The cutoff can be adjusted to the concentration of the low control (0.4 ng/mL), which would detect carfentanil in these cases.

Keywords: Carfentanil, immunoassay, fentanyl analogues.

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Identification and Separation of Opioid Metabolites in Urine by LC-MS/MS

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Background/Introduction: With the recent addition of oxycodone, oxymorphone, hydrocodone and hydromorphone to the October 2017 Mandatory Guidelines for Federal Workplace Drug Testing Programs, it is imperative that samples containing such drugs are accurately tested by laboratories. Chromatographic separations are essential for the analysis and correct identification of opioids by LC-MS/MS since some of the compounds are structural isomers and the metabolites can generate the same fragments in the mass spectrometer. In particular, our lab found that samples containing oxycodone and oxymorphone have interfering peaks detected by LC-MS/MS which make accurate quantitation of codeine, morphine and hydromorphone challenging.

Objective: To identify opioid metabolites found in patient samples that can lead to possible misidentifications of codeine, morphine, hydrocodone, hydromorphone and dihydrocodeine by LC-MS/MS analysis.

Method: Fifteen different opioid metabolites were diluted in negative human urine and prepared for analysis by enzymatic hydrolysis. The samples were then injected into a Shimadzu Nexera UHPLC system equipped with a Restek Raptor Biphenyl column. Detection was performed using a Sciex API6500 QTRAP with electrospray ionization in positive mode. Analytical separations were evaluated by using different columns of varying stationary phases and lengths.

Results: All opioid metabolites were baseline resolved from our analytes of interest with the use of a 100mm biphenyl column. The potentially interfering compounds evaluated were norcodeine, noroxycodone, norhydrocodone, noroxymorphone, 6-AM, 6α-oxycodol, 6β-oxycodol, 6α-oxymorphol, 6β-oxymorphol, nor-6α-oxycodol, nor-6β-oxycodol, 6α-oxycodol N-oxide, 6β-oxycodol N-oxide, 6β-hydrocodol and 6β-hydromorphol. We identified the oxycodone metabolites observed in patient samples as 6α-oxycodol and 6β-oxycodol. These compounds form fragments that interfere with the analysis of codeine(300→215m/z, and 300→165m/z) by generating near-coeluting peaks with similar ion ratios; it was also noted that oxycodone/oxymorphone metabolites, 6α-oxymorphol and 6β-oxymorphol, form fragments that interfere with the analysis of morphine(286→201m/z, and 286→165m/z) by generating near-coeluting peaks with similar ion ratios; and oxycodone metabolites, nor-6α-oxycodol and nor-6β-oxycodol and norcodeine, form fragments that interfere with the analysis of hydromorphone(286→185m/z, and 286→157m/z) by generating near-coeluting peaks with similar ion ratios. All other columns evaluated were not able to fully resolve 6α-oxycodol and 6β-oxycodol from codeine.

Conclusion/Discussion: The analysis of opioids and their metabolites by LC-MS/MS can be challenging and difficult to accomplish in routine human samples. With oxycodone metabolizing to noroxycodone (<47%), oxymorphone (11%), oxycodol (8%) and less than 10% excreted unchanged the relative amount in samples is significant. When testing for opioids it is best practice to use longer columns, run a slower gradient, and monitor the relative retention times of your compounds due to the similar metabolite fragmentation patterns. By identifying interfering compounds seen in patient samples, a more robust method was developed to ensure accurate analytical results with easier data review by separating metabolites of related compounds.

Keywords: LC-MS/MS, Opioids, Metabolites

POSTER

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Development of a Highly Sensitive ELISA for Detection of α-PVP, MDPV and Closely Related Bath Salts in Urine

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Introduction: -pyrrolidinopentiophenone or -pyrrolidinovalerophenone (-PVP) is a designer stimulant belonging to the synthetic cathinone class of drugs. It is commonly known by the street name "flakka" in Florida or "gravel" in other parts of the United States and has been identified as an ingredient in mixtures commonly sold as "bath salts". 3,4-methylenedioxypyrovalerone (MDPV) is its structurally related analogue. Both compounds are DEA schedule I in the US, along with several other cathinones. Several related compounds including -PVP are banned substances in many other countries as well. Synthetic cathinones are typically snorted, smoked or ingested. These substances are norepinephrine-dopamine reuptake inhibitors that may prompt a reaction termed "excited delirium" involving erratic behavior, hallucinations, delusions and in some cases also cause death. In recent years there has been a significant increase in the number of such designer drugs that are being abused. The 2013-2015 NFLIS report cited the 20 most frequently reported synthetic cathinones, which included -PVP at 28.9% (second highest) and MDPV at 3% of the total, or roughly 14,995 and 1,608 cases in the US respectively.

Objective: To develop a highly sensitive ELISA for the detection of -PVP, MDPV and structurally related designer stimulant bath salts in urine.

Method: A new polyclonal antibody to -PVP was developed and immobilized on a microtiter plate. Calibrators were prepared by fortifying -PVP in synthetic negative urine at concentrations of 0.1, 0.25, 0.5, 1, 2, 5 and 10 ng/mL. The calibrators and urine specimens were then diluted 1:10 with 0.1M phosphate buffered saline (PBS). The calibrator set (25 µL, 4 replicates) and urine specimens (25µL, 2 replicates) were pipetted on the -PVP microtiter plate, followed by -PVP enzyme conjugate (100 µL). The plate was incubated for 60 minutes at ambient temperature, then washed 6 times with deionized water. Enzyme substrate was added to the plate and incubated for 30 minutes at ambient temperature. The reaction was stopped with 1N hydrochloric acid and read at dual wavelengths of 450 and 650 nm, using a Tecan microplate reader. The absorbance signal vs. concentration was plotted to obtain the dose-response curve. Various designer cathinones were also fortified in synthetic negative urine at multiple concentrations and screened by ELISA, to determine their cross-reactivity at the assay cutoff.

Results: The ELISA is capable of detection of -PVP at a low cutoff of 0.5 ng/mL in urine. The limit of detection (LOD) of the assay is 0.1 ng/mL. The antibody developed shows 100% cross-reactivity to MDPV and pyrovalerone. The assay also shows a high cross-reactivity to naphyrone (300%), 5-DBFPV (400%), -PVT (100%), -PBP (40%), 3,4-MDPBP (50%), 4-methyl PBP (20%) and 4-methyl PHP (50%). This assay showed no cross-reactivity to synthetic cathinones like methylone, ethylone or butylone at the assay cutoff, due to absence of a pyrrolidine ring structure which is present in -PVP. Common prescription and illicit drugs at fortified concentrations up to 100,000 ng/mL in synthetic negative urine were also screened for cross-reactivity in the assay, but none was observed. The assay was validated with 60 authentic urine specimens.

		GC-MS	
		+	-
ELISA	+	30	0
	-	0	30

30 true positive specimens containing -PVP ranging from 40-17,000 ng/mL were screened by ELISA. 30 specimens were true negatives by both ELISA and GC-MS. The GC-MS confirmation profile run by an external source, included multiple bath salts, i.e. -PVP, methylone, ethylone, butylone and pentylone (25 ng/mL) and MDPV (50 ng/mL) at the cutoffs indicated.

Conclusion: A highly sensitive ELISA for -PVP, MDPV, pyrovalerone and several closely related designer cathinones at a cutoff of 0.5 ng/mL in urine has been developed. This assay would enable toxicology laboratories to screen for these synthetic cathinones in urine. Further application of this work could be to extend it to the detection of these compounds in blood and oral fluid.

Keywords: -PVP, MDPV, Bath Salts

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Development of a Highly Sensitive Polyclonal Antibody for Detection of PB-22, 5F-PB-22 and Related Synthetic Cannabinoids in Oral Fluid by ELISA

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Introduction: Designer synthetic cannabinoid trends have been rapidly changing over the last few years. The chemical modifications possible that allow these compounds to bind the CB_1 and CB_2 cannabinoid receptors have resulted in constant new generations of designer compounds that stay ahead of Drug Enforcement Agency (DEA) scheduling attempts. PB-22 and 5F-PB-22 (DEA Schedule I substances) and BB-22, while still retaining the core JWH-018 indole ring structure, contain an ester-linked quinoline substructure. The National Forensic Laboratory Information System (NFLIS) report from 2013-2015 indicated PB-22, 5F-PB-22 and FUB-PB-22 constituted slightly more than 8% (8,167 cases in the US) of the 25 most frequently reported synthetic cannabinoids. We have previously demonstrated the utility of oral fluid in the detection of numerous parent therapeutic drugs and drugs of abuse. The Quantisal™ oral fluid collection device allows observed collection of a fixed amount of saliva sample, thereby preventing adulteration of specimen. We have also previously published studies showing the detection of parent synthetic cannabinoids, i.e. JWH-018 and UR-144 in oral fluid by ELISA.

Objective: To develop a highly sensitive polyclonal antibody for detection of the parent synthetic cannabinoids: PB-22, 5F-PB-22, FUB-PB-22 and BB-22, for application to an oral fluid ELISA.

Method: Using the Quantisal™ collection device, 1 mL of oral fluid specimen is collected. The device then dilutes the specimen with 3 mL of oral fluid stabilization buffer. A PB-22 polyclonal antibody that was developed by immunization of sheep with an appropriate PB-22 antigen, was immobilized on a microtiter plate. PB-22 oral fluid calibrators (75µL) at concentrations of 0.25, 0.5, 1, 5, 10, 25 and 50 ng/mL and oral fluid specimens (75µL) fortified in Quantisal™ buffer were pipetted in duplicate on the microtiter plate and allowed to pre-incubate for 30 min. This was followed by addition of PB-22 enzyme conjugate (50µL) and incubated for a further 60 min. The plate was washed with deionized water and then incubated with enzyme substrate (30 min). The reaction was stopped with 1N HCl and read at 450 and 650 nm, using a Tecan microplate reader. Cross-reactivity studies on the antibody were performed by fortifying oral fluid buffer with different generations of synthetic cannabinoids, as well as other illicit and prescription drugs.

Results: This polyclonal antibody is highly sensitive, capable of detecting PB-22 at a neat oral fluid cutoff concentration of 1 ng/mL. The assay is highly cross-reactive towards 5F-PB-22 (306%), FUB-PB-22 (86%) and BB-22 (68%). No cross-reactivity was detected with any of the other generations of synthetic cannabinoids, i.e. JWH-018, JWH-250, UR-144 or AB-PINACA up to 100, 000 ng/mL. No other illicit/prescription drugs (100,000 ng/mL) cross-reacted with the assay. Due to some difficulty in obtaining authentic oral fluid specimens, validation of the antibody was accomplished by fortifying authentic negative oral fluid with PB-22, 5F-PB-22 and BB-22, ranging from 0-1000 ng/mL. All specimens screened correctly by ELISA, and the assay only did not detect BB-22 when fortified at 0.2 ng/mL, which was below the assay detection limit.

Conclusion: The specific design of the antigen used to raise the polyclonal antibody allows it to recognize differences in molecular structure, thereby allowing it to selectively detect PB-22 and very closely related compounds, but not other generations of synthetic cannabinoids. This highly sensitive antibody can be utilized in an ELISA for the detection of the synthetic cannabinoids: PB-22, 5F-PB-22, FUB-PB-22 and BB-22, using a neat oral fluid cutoff concentration of 1 ng/mL. Although no authentic specimens were obtained, perhaps toxicologists might be encouraged to utilize oral fluid as a matrix to screen for these parent synthetic cannabinoids.

Keywords: PB-22, 5F-PB-22, Oral Fluid

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A Modified QuEChERS Approach for the Extraction of Benzodiazepines from Blood Prior to LC-MS/MS Analysis

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Background/Introduction: Benzodiazepines (Benzos) are psychoactive drugs widely prescribed for treating anxiety, insomnia, agitation, seizures, muscle spasms, and alcohol withdrawal. Benzos are deemed safe and effective for short term use. However, frequent use of these drugs may lead to dependence and abuse. Many clinical, forensic, and toxicological laboratories are interested in monitoring these compounds in biological samples for this reason. Common sample preparation methods for biological samples include a protein precipitation step followed by liquid-liquid extraction (LLE) or solid phase extraction (SPE). While these two approaches work well, they often call for the need for several solvents and lengthy procedures to execute. This study explores a modified QuEChERS approach for the quantitative analysis of benzodiazepines in whole blood.

Objective: To evaluate an alternative method for the extraction of benzodiazepines from blood prior to analytical analysis by LC-MS/MS.

Method: Blank blood (1 mL) was fortified with appropriate amounts of working standards prior to being added to 15 mL centrifuge tubes containing QuEChERS salts (400 mg magnesium sulfate (MgSO4) and 100 mg sodium acetate (NaOAc) and 2 mL of acetonitrile containing 5% formic acid. Samples were briefly vortexed to break up any salt agglomerates prior to shaking for 5 minutes at a rate of 1000 strokes/minute using a SPEX Geno/Grinder®. After shaking, samples were placed into a centrifuge and spun for 10 minutes at a speed of 4000 rpm. Further sample cleanup was performed by adding 1 mL of the centrifuged supernatant to 2 mL micro-centrifuges tubes containing 150 mg of MgSO4, 50 mg of PSA (primary secondary amine), and 50 mg endcapped, silica based C18 sorbent. Samples were vortexed at a rate of 100 strokes/minute for 1 minute, then placed into a centrifuge and spun for 5 minutes at a rate of 4000 rpm. 400 µL of purified sample was transferred to autosampler vials containing 400 µL, vials were briefly vortexted and transferred to the LC-MS/MS for analysis. Matrix effects were evaluated by comparing the slopes of the matrix matched calibration curves to those of the calibration curves of solvent standards. Recoveries were calculated by dividing the chromatographic peak area of samples spiked prior to extraction by the peak area produced by samples that were spiked into a pre-extracted blank matrix

Results: Matrix matched calibration curves were constructed for the benzodiazepines quantification. The responses for 10 representative compounds were linear with R^2 ranging from 0.9963 to 1.0000 over the concentration range of 10 - 500 ng/mL. The matrix effects were found to be minor, ranging from -22 to 18%. Excellent recoveries (85.5 - 105%) and relative standard deviations (RSD% ≤ 10.7%) were obtained.

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Compound	Spiked at 10 ng/mL		Spiked at 50 ng/mL		Spiked at 200 ng/mL	
	Recovery%	RSD%	Recovery%	RSD%	Recovery%	RSD%
7-Aminoclonazepam	88.6	7.5	96.9	2.1	99.7	3.8
Alpha-Hydroxy-Alprazolam	101.2	3.4	91	2	90.3	2.7
Alprazolam	92.3	10.7	90.2	4	86.5	3.5
Clonazepam	96.4	3.6	105	3.2	103	2
Diazepam	85.5	3.3	103	2.7	100.4	1.9
Lorazepam	96.9	5.1	93.7	4.1	91.6	2.7
Midazolam	96.7	2.7	101.6	2.7	100.6	1.9
Nordiazepam	88.4	3.9	99.7	2.5	97.8	2.3
Oxazepam	86.5	1.9	93.8	2.4	92.6	1.7
Temazepam	96.7	2.7	101.6	2.7	100.6	1.9

Conclusion/Discussion:

Preliminary studies have shown that this modified QuEChERS-based method for the extraction of benzodiazepines from blood should be considered as a reliable alternative when extracting multiple drug classes from challenging biological matrices.

Keywords: QuEChERS, Benzodiazepines, Blood

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Simultaneous Quantitative Analysis of Total Catecholamines and Metanephrines in Urine Using SPE and LC-MS/MS

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Background/Introduction: Patients who are thought to have a pheochromocytoma, a condition in which a neuro-endocrine tumor develops in the medulla of the adrenal glands, are often diagnosed following biochemical tests that measure the levels of catecholamines and metanephrines. The structures and pKa values of epinephrine, norepinephrine, metanephrine, normetanephrine and dopamine make them ideal candidates for clean-up via strong cation exchange solid phase extraction (SPE). Initial evaluations of this approach produced excellent recoveries of the metanephrines. However, it greatly reduced the recovery of the catecholamines regardless of the wash/elution solvent combination. Further investigation suggested that the use of highly basic elution solvents containing ammonium hydroxide led to degradation of the catecholamines. The approach was changed from using a strong cation exchange sorbent to a weak cation exchange sorbent. The weak cation exchange approach allowed for the use of an acid elution solvent rather than a basic one which resulted in excellent recoveries of both classes and prevented the degradation of the catecholamines.

Objective: To develop a high throughput method to simultaneously extract catecholamines and metanepherines from urine using SPE prior to LC-MS/MS analysis.

Method: Samples were prepared by adding 3 mL of 100 mM phosphate buffer (pH 7.0) and internal standards to 1 mL of urine samples. Once sample pre-treatment was completed, the extraction columns were conditioned with 3 mL of methanol (MeOH), followed by 3 mL of deionized water, and finally 3 mL of 100 mM phosphate buffer (pH 7.0). Samples were then applied to the SPE cartridges and were allowed to flow through the columns at a rate of 1 mL/minute. Columns were washed with 3 mL of deionized water followed by 3 mL of a 50/50 mixture of methanol/acetonitrile. After drying the cartridges for 10 minutes under full positive pressure, analytes were eluted with 3 mL of methanol containing 5% formic acid. 100 µL of 1% HCl in MeOH was added to the samples to prevent volatilization by the formation of the hydrochloric salt of the compounds. Samples were then evaporated to dryness and reconstituted with 100 µL of mobile phase. Recoveries were evaluated by fortifying samples at three varying concentrations. Data was calculated by dividing the chromatographic peak areas of urine samples spiked prior to extraction by those that were spiked post-extraction.

Results: Excellent recoveries were achieved for the range of analytes included in this study. On average, the recovery for samples spiked at 25 ng/mL was 82.5%, for samples spiked at 500 ng/mL it was 78% and for samples spiked at 2000 ng/mL it was 70%.

Conclusion/Discussion: Preliminary studies have shown that a weak cation solid phase extraction method for the simultaneous extraction of catecholamines and metanephrines from urine should be considered as a reliable approach when testing these hormones in biological samples. This combined method allows analysts to report results not only accurately, but also faster than having two separate methods.

Keywords: Catecholamines, Metanephrines, SPE

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Picogram Detection of the Marijuana Metabolite THC-COOH in Hair Samples using an Efficient and Sensitive LC-MS/MS Analysis Workflow

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Background/Introduction: Marijuana is one of the most popular recreational drugs abused worldwide. Detection of its use can be done through biological matrices like urine, oral fluid, and hair. While urine and oral fluid are very useful for determining marijuana use in short term, hair samples are extremely valuable in testing the long-term use. Nonetheless, low metabolite concentration and high matrix interferences are major analytical challenges associated with detecting active marijuana abuse through hair samples.

Objective: In this presentation, a complete analysis workflow that includes triple quadrupole mass spectrometry in combination solid phase extraction for the efficient and sensitive detection of picogram levels of THC-COOH in hair matrix is described.

Methods: *Sample Preparation:* Hair samples were washed, dried and cut into segments of ~ 2 mm lengths and spiked with deuterated internal standard (IS). Alkaline digestion was performed for 1 hour, followed by ultra-centrifugation. Supernatants subsequently passed through Strong Anion Exchange (SAX) Solid Phase extraction cartridges to selectively trap and elute THC-COOH from the matrix. Sample Extracts were reconstituted in mobile phase for LC-MS/MS analysis. *LC-MS/MS Conditions:* HPLC separation was performed using a Phenomenex Kinetex phenyl-hexyl (50 x 3 mm, 2.6 µm) column. Mobile Phase was acetic acid in water and methanol, 750 µL/min flow rate. A SCIEX Triple Quad™ 4500 LC-MS/MS operating in negative electrospray mode was used for detection, using two MRM transitions for THC-COOH and IS.

Results: One of the biggest challenges encountered in this study was obtaining clean extracts from hair samples due to the presence of complex matrix contents, interfering with the detection THC-COOH. . To remove these interferences, a SAX SPE procedure was suggested and tested. It was discovered that the samples required alkalinization with Potassium Hydroxide before being applied to the SPE cartridge, which ensured the selective extraction of THC-COOH by the oppositely charged SAX stationary phase.

Sample preparation recovery and matrix effects were tested by preparing three different control hair sample sets. It was observed that the sample preparation recovery was at 68% and the matrix effects showed 22% loss of signal (or 78% signal recovery due to ion suppression). This allows the reliable quantitation of THC-COOH at low picogram concentration levels, which is only possible through the implementation of the SAX SPE procedure designed. The LC-MS/MS analysis workflow combined with SAX SPE provided a Lower Limit of Quantitation (LLOQ) for THC-COOH in hair matrix of 0.2 pg/mg, Linear Dynamic Range of the method was evaluated between the LLOQ and 2 pg/mg. The workflow showed excellent accuracy (>95%) and precision (< 15%), with excellent linearity resulting in R2 values for quantifier ion (343→245) and qualifier ion (343→191) of 0.9987 and 0.9983, respectively.

Conclusion/Discussions: The combination of SPE SAX with LC-MS/MS allowed the efficient and sensitive detection of trace levels of THC-COOH (0.2 pg/mg) in hair samples, making the workflow to be readily adaptable into a forensic laboratory. The application of the SCIEX Triple Quad™ 4500 LC-MS/MS system was demonstrated to provide unique advantages when confirming and quantifying low level metabolites in hair when utilized with Solid Phase Extraction.

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

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Ultra-Sensitive Forensic Analysis Workflow of Cocaine and Metabolites in Hair Samples Using LC-MS/MS

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Background/Introduction: Forensic hair analysis for screening and confirmation of drugs of abuse has become a valuable tool for toxicologists, as it provides an extended window of detection compared to other possible matrixes. To confirm cocaine use, the detection of cocaine and/or its metabolites is required, which subsequently necessitates a sensitive and reliable analytical method. There are two major challenges for the detection of cocaine and its metabolites in hair samples: low concentrations and matrix interferences.

Objective: This presentation describes the use of a linear ion trap mass spectrometry and solid phase extraction (SPE) for the picogram detection of cocaine and metabolites in hair.

Methods: *Sample Preparation:* 20 mg Hair samples were washed, dried and cut into segments of ~ 2 mm lengths and spiked with 0.25 pg/mg deuterated internal standard solution. The analytes were extracted with 0.1N HCl via sonication for twenty-four hours. Supernatants were subsequently passed through Phenomenex Strata-X SPE cartridges, dried and reconstituted in mobile phase for LC-MS/MS analysis. *LC-MS/MS Conditions:* HPLC separation was performed at 30 ºC on a Phenomenex Kinetex biphenyl column (100 × 3.0 mm, 2.6µm). Mobile Phase was water and methanol with 0.1% formic acid, 600 µL/min flow rate using a gradient with a total run time of 10 min. A SCIEX QTRAP® 6500+ LC-MS/MS system operating in positive electrospray mode was used for detection, using two MRM transitions for cocaine, benzoylecgonine, ecgonine methyl ester, cocaethylene, norcocaine, ecgonine, m-OH cocaine, o-OH cocaine, p-OH cocaine, m-OH BZE, and p-OH BZE Deuterated standards used in this work included: Benzoylecgonine-d3, Cocaethylene-d3, Cocaine-d3, and Norcocaine-d3.

Results: One of the main challenges encountered in this study was the reliable detection and quantitation of cocaine and metabolites in hair, due to the presence of complex matrix contents. To remove these interferences, Phenomenex Strata-X SPE cartridges were used to selectively isolate the cocaine and its metabolites. Extraction recoveries were greater than 80% while matrix effects showed more than 65% signal recovery due to ion suppression. The LC-MS/MS analysis workflow combined with SPE enabled sub pg/mg Lower Limits of Quantitation in hair matrix. The method showed excellent accuracy (>95%) and precision (< 15%), with good linearity 0.1-1000 pg/mg, resulting in R^2 values of 0.9990 for all analytes.

Conclusion/Discussions: A method for the simultaneous determination of cocaine and metabolites at picogram levels was developed and evaluated in hair matrix. Utilization of the SCIEX QTRAP® 6500+ LC-MS/MS system was demonstrated to maximize linear dynamic range when selectivity when confirming and quantifying low level metabolites in hair. In addition to the quantitation of cocaines, the SCIEX QTRAP® 6500+ LC-MS/MS system enabled simultaneous identification and confirmation of other illicit drugs and their metabolites through utilization of Enhanced Product Ion Scan (EPI) by acquiring full scan MS/MS data. Forensic drug identification and confirmation was achieved using automated MS/MS library searching.

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

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Streamlined Forensic Postmortem and DUI Drug Screening using High Resolution Mass Spectrometry

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Background/Introduction: Accurate identification of drugs present in postmortem and/or DUI case samples is crucial for forensic toxicologists to successfully carry out an investigation. The identification of these compounds often raises important questions and may provide immediate answers about cause of death and other related antemortem events. Traditionally, drug screens are either done by immunoassay or GC/MS. Immunoassay techniques are often not conclusive enough (false positives or cross-reactive compounds) or have limited scope. GC/MS requires sample derivatization and lengthy chromatographic runs to accurately identify Novel Psychoactive Substances (NPS) and other drugs present in a forensic sample. The implementation of High Resolution Mass Spectrometry (HRMS) instrumentation in the forensic laboratory helps toxicologists to rapidly obtain complete chemical profiles from biological samples, (e.g., acquisition of analyte specific MS/MS spectra), increasing confidence in compound identification at low concentration levels.

Objective: In this presentation, a comprehensive drug screening workflow for the analysis of forensic blood samples is described. The analysis was streamlined using a simplified sample preparation approach in combination with SWATH® Acquisition and MRM^{HR} workflow on the SCIEX X500R QTOF System.

Methods: *Sample Preparation:* Control whole blood samples were spiked with a stock standard solution mixture containing 155 different drugs for the initial method development. Forensic case postmortem blood samples were extracted using a protein precipitation procedure. The supernatant was transferred out and completely dried down under nitrogen gas, followed by reconstitution and injection. *LC-HRMS Conditions:* HPLC separation was performed at 30 °C on a Phenomenex Kinetex Phenyl-Hexyl column (50 × 2.1 mm, 2.6µm) on the SCIEX ExionLC™ AC system. Mobile phases used were water and acetonitrile with appropriate buffer additives. The LC flow rate was 0.5 mL/min and the LC runtime was 7 minutes in positive electrospray mode and 5.5 minutes in negative electrospray mode. MS and MS/MS data were collected using SWATH® Acquisition and MRM^{HR} Workflow on the SCIEX X500R QTOF System with SCIEX OS Software.

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Results: A SCIEX vMethod™ Application for 664 forensic compounds with default separation conditions was initially installed and further optimized to maximize compound coverage for forensic postmortem and DUI case samples. Information-dependent acquisition, was initially applied to acquire and store MS/MS spectra for each target compound of interest. Nonetheless, it was found that the comprehensive nature of SWATH® Acquisition enabled the possibility of obtaining unrestricted fragment ion spectra generation over the whole run, minimizing the risk of missing potential forensic compounds present (e.g., NPS) in blood samples. Thus, giving the option to comprehensively re-interrogate the sample data should new questions arise in the future. On the other hand, MRM^{HR} workflow enabled the sensitive detection of negative electrospray mode ionizable compounds (e.g., 5 ng/mL THC-COOH) in forensic DUI and postmortem blood samples. This targeted data acquisition strategy used the selective high resolution, accurate mass MS/MS information as well as spectral library matching for identification purposes.

Conclusion/Discussions: A comprehensive drug screening workflow for the analysis of postmortem blood samples has been developed using the SCIEX X500R QTOF System. SWATH® Acquisition generated comprehensive and high-quality MS/MS spectra, enabling reliable compound fragmentation comparison to library spectra for confident drug identification and retrospective analysis to avoid missing potential NPS present in both forensic postmortem and DUI case samples. MRM^{HR} workflow enabled the sensitive detection of common compounds that better ionize using negative electrospray mode.

Keywords: Postmortem Screening, LC-HRMS, DUI

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Single-Injection Screening of 664 Forensic Toxicology Compounds using an Innovative Benchtop High Resolution Mass Spectrometer

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Background/Introduction: Quadrupole Time-of-flight mass spectrometry (QTOF-MS) provides high-resolution, accurate-mass data for full-scan information of both precursor ion and all product ions. This is an ideal approach for forensic toxicology screening where unknown compounds in complex biological samples must be identified from information-rich data sets. Due to its straightforward design and novel software workflows, non-targeted data obtained on a QTOF-MS system can be retrospectively mined for additional analytes missed in initial screens, which is important with the constant emergence of new synthetic drugs.

Objective: In this presentation, a single-injection method for screening 664 most up-to-date forensic compounds using an innovative benchtop QTOF mass spectrometer is described. The obtained data provided both structural information and retention times to enhance identification accuracy, especially for structurally similar isomers. Sample preparation procedures for urine and whole blood samples and library-search settings are described for confident unknown substance identification within an efficient, all-in-one workflow.

Methods: *Sample Preparation:* Urine and whole blood samples were spiked with stock standard mixtures and used to determine the retention time of the 664 compounds. Urine samples were diluted with mobile phase and analyzed; whole blood samples, were extracted by using protein precipitation and centrifugation; supernatant was evaporated and reconstituted in mobile phase for analysis. *LC-HRMS Conditions:* Analytes were chromatographically separated using a Phenomenex Kinetex phenyl-hexyl (50 x 4.6 mm, 2.6 µm) column. Mobile Phase was ammonium formate in water and formic acid in methanol, 600 µL/min flow rate. The QTOF-MS was operated in positive electrospray mode with information dependent acquisition MS/MS methods. Samples were evaluated against a list of parameters containing the names, molecular formulas and retention times for all compounds.

Results: The performance of separation was evaluated with different mobile phases (acidic and neutral), gradient conditions, and column types. Results indicate that most of isomeric compounds were fully resolved with neutral Buffer A and a 10-min linear gradient using the phenyl-hexyl column. Analyte retention time (RT) was a critical element for accurate identification of each forensic analyte using this screening method, the following RT reproducibility tests were conducted for each compound to evaluate the robustness of the LC condition in this method: (1) reproducibility on 3 separate columns; (2) the inter-day (n=3) reproducibility; (3) the reproducibility in neat versus matrix samples. The reproducibility tests indicated that the RTs generated from the optimized LC conditions are consistent and reproducible. RTs measured on three separated analytical columns all have %CVs of less than 5% for each of the 664 compounds. RT inter-day reproducibility (tested on 80 compounds) resulted in %CVs less than 5% over 3 days. Lastly, RT variability in human whole blood and urine samples (tested on 80 compounds) indicated that the %CV for 3 individual lots is less than 5%. The retention time determined by the optimized LC condition combined with high-resolution mass spectrometry and MS/MS spectra, enabled accurate compound identification across the workflow. Retrospective analysis was also performed on the acquired data sets to screen for new compounds without having to re-inject samples, allowing data sets to be re-processed as new forensic targets were discovered.

Conclusion/Discussions: An LC-MS/MS-based screening method that includes the Retention Times for 664 forensic compounds has been developed. When combined with high-resolution mass spectrometry the retention time identified herein enable more accurate compound identification. In addition, the data was acquired in a non-targeted approach enabling unknown compound identification through retrospective analysis.

Keywords: Comprehensive Drug Screening, Urine and Whole Blood, LC-HRMS

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Analytical evaluation of Randox ELISA kits for a reliable comprehensive routine drugs of abuse screening in urine and blood

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Background/Introduction: Enzyme-linked immunosorbent assays (ELISAs), due to the ease of use, potential automation and application to different matrices, are useful analytical tools in forensic toxicology for the screening of drugs of abuse. Assay sensitivity is important to consider in the screening step, and assay accuracy and precision are analytical parameters that ensure reliability of the sample assessment.

Objective: This study presents the analytical evaluation of twelve Randox ELISA kits for the screening of drugs of abuse including analgesics, stimulants, hallucinogens, and tricyclic antidepressants in urine and blood. Assay sensitivity, accuracy and precision were evaluated to ensure optimal routine drugs of abuse screening results.

Method: Twelve Randox ELISA Kits were used for the routine drugs of abuse screening in urine and blood. The assays and the cut-offs (for urine and blood respectively) were as follows (for some assays in urine there are two possible cut-offs): amphetamine 300/500 ng/mL and 50 ng/mL, barbiturates 200 ng/mL and 50 ng/mL, benzodiazepines 200 ng/mL and 10 ng/mL, benzoylecgonine 300/150 ng/mL and 50 ng/mL, cannabinoids 50ng/mL (urine and blood), meprobamate 50ng/mL (urine and blood), methadone 300 ng/mL and 10 ng/mL, methamphetamine 300/500 ng/mL and 50 ng/mL, phencyclidine 25 ng/mL and 5 ng/mL, opiates 300 ng/mL and 25 ng/mL, oxycodone 10 ng/mL (urine and blood), tricyclic antidepressants 100 ng/mL and 25 ng/mL.

The limit of detection (LOD) was determined by the assessment of negative urine or blood samples (n=20). The mean concentration was calculated and 3 standard deviations added.

Recovery was determined by preparing spiked samples in negative matrix with the target material at three levels. The concentration of these samples was determined from the standard curve and expressed as percentage recovery of the expected concentration (n=12).

Intra-assay precision was determined by assessment of replicates (n=12) of different concentration levels for each target drug. Results were expressed as CV (%).

Results:

The LODs (ng/mL) in urine (URN) and blood (B) were as follows:

Analyte	d-Amphetamine	Phenobarbital	Diazepam	Benzoylecgonine
LOD URN/B	108.2/11.7	42.7/17.3	32.64/6.62	1.12/2.96
Analyte	**(-)-11-nor-9-carboxy-Δ9-THC**	**Meprobamate**	**Methadone**	**(+) Methamphetamine**
LOD URN/B	17.2/23.9	4.78/7.64	1.85/0.18	37.7/0.0
Analyte	**Phencyclidine**	**Morphine**	**Oxycodone**	**Nortriptyline**
LOD URN/B	0.79/0.69	5.53/1.15	2.08/3.85	33.6/10.9

The analytical evaluation showed a typical recovery range of 82%-129% (urine) and 70%-130% (blood). The intra-assay precision was ≤10% for all the assays when different concentration levels of the target drug were assessed.

Conclusion/Discussion: Data show optimal analytical performance of the twelve ELISA kits evaluated, which indicates their applicability to the reliable screening of a broad range of drugs of abuse including analgesics, stimulants, hallucinogens, and tricyclic antidepressants.

Keywords: ELISA, Routine drug screening, Drugs of abuse

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Development of High Throughput Screening Method for ETG and Creatinine in Urine using LDTD-MS/MS

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Background/Introduction: Ethyl glucuronide (ETG) is a metabolite of ethanol usually used as a biomarker to test urine for ethanol ingestion. The creatinine concentration in urine is quantified to verify the sample's integrity. Normal creatinine levels indicate that the test sample is undiluted.

For a fast screening of both analytes, a quick and simple sample preparation was developed, and quantification was performed using Laser Diode Thermal Desorption mass spectrometry (LDTD-MS/MS).

Objective: Using a quick sample preparation, perform quantification of ETG and creatinine on a LDTD-MS/MS screening system. Evaluate matrix interferences and reproducibility around the cut-off concentration.

Method: Varying concentrations of ETG (500 ng/mL to 4000 ng/mL) were spiked in negative urine to determine the cut-off concentration using the two standard deviations approach (2SD). For creatinine, quantification against a stable label analogue was used for the determination of endogenous concentration.

For the validation experiment, 5µL of spiked sample was diluted with 995 µL of internal standard solution (400 ng/mL of creatinine-d3 and 50 ng/mL ETG-d5) in a mixture of methanol:water:MTBE. Four microliters of diluted solution were deposited on a LazWell-AD plate and evaporated to dryness. Samples were transferred to the mass spectrometer using a six second laser ramp to 65% power using the LDTD system. The mass spectrometer was operated in negative mode using the following transitions: 112→68 (Creatinine), 115→68 (Creatinine-d3), 221→75 (ETG), 226→75 (ETG-d5).

Results: For ETG, the 2SD approach was used for the cut-off determination. At 500 ng/mL, no error bar overlap was observed against the blank. The curve was linear between 500 and 4000 ng/mL(r=0.9954). Quantification of creatinine was performed against the stable label creatinine-d3. The reproducibility experiment of creatinine evaluation gave a mean value of 432 mg/L with a %RSD of 7.6% (n=36). Twenty real samples were analyzed using the screening procedure and compared to the results obtained using a LC-MS/MS confirmation method. The creatinine sample concentration values were between 233 and 1445 mg/L. The comparison plot of LDTD-MS/MS and the LC-MS/MS method gave a $r^2 > 0.97$. For the LDTD-MS/MS screening analysis of ETG, 13/20 real samples were detected as positive. The same samples were analyzed using the LC-MS/MS method and all positive samples were confirmed.

Conclusion/Discussion: A simple 200 times dilution of urine was used for a quick screening of ETG and creatinine quantification. Acceptable reproducibility and accuracy were obtained. All sample values were cross validated with a LC-MS/MS confirmation method. LDTD-MS/MS allowed fast quantitation of ETG and creatinine in urine.

Keywords: ETG, Creatinine, LDTD-MS/MS

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Development of Low Blood Volume (15 µL) Collection Device for THC Analysis and Quantification Using LDTD-MS/MS

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Background/Introduction: Δ^9-tetrahydrocannabinol (THC) is the major psychoactive component of cannabis. When consumption ends (smoked), THC levels in blood decrease rapidly, giving a short period of time for police enforcement to collect blood. Therefore, a low volume collector is developed using the Dry Blood Spot (DBS) card approach.

Objective: A new collection device is developed for fast and low blood collection volume for THC analysis. Using the LDTD-MS/MS system for quantification of THC, the precision, accuracy and stability of the blood collection device are evaluated.

Method: Whatman 903 cards (5 mm X 5 mm) are mounted on a stainless-steel stick. The device is then used to collect 15 µL of blood directly after a finger puncture. The card piece is then transferred into a closable vial containing a buffer solution and sent to a laboratory for analysis. Quantification using Laser Diode Thermal Desorption mass spectrometry (LDTD-MS/MS) is chosen as a fast-analytical technique that requires low sample volumes. The card size is optimized to collect 15µL of blood. After blood collection, card portions are transferred into a glass tube containing 100µL of EDTA buffer. For the validation experiment, 15µL of blood spiked with THC are added on the device. 10µL of internal standard solution (THC-d3) are added in a tube then vortexed. 100µL of extraction solution (Hexane:Ethyl acetate / 90:10) are used for the liquid-liquid extraction. 8µL of the upper phase are deposited on a LazWell plate and evaporated to dryness. Samples are transferred to the mass spectrometer using a 6 second laser ramp to 45% power. The mass spectrometer is operated in negative mode using 313→245 transition for THC.

Results: For the blood volume absorption precision and accuracy experiment, negative blood is spiked with THC at 25 ng/mL. Approximately 25 µL of the test sample are added on a glass surface to simulate a finger puncture. The blood is then collected with a card until complete surface absorption. To evaluate the THC concentration and confirm blood absorption volume, 15 µL of spiked blood is added on the same type of card using an automatic pipette.

The calibration curve is linear over concentrations ranging from 2.5ng/mL to 100 ng/mL. The coefficients of determination (R^2) are greater than 0.99 for the quantification curve of THC. The precision and accuracy of Lower Limit of Quantification (LLOQ) are 9.6% and 99.7% for the inter-day experiment (n=12). All precision values are within 2.5 to 9.6% and accuracy values are within 98.2 to 102.1%. After blood collection, card devices are transferred in the extraction tube containing buffer and cap. Tubes are then stored at 4°C for 18h. After the stability time, THC is extracted and analyzed. Stability samples are evaluated against a fresh spiked calibration curve and good stability accuracy of 89.8% with precision of 8.0% are reached. For the blood volume absorption precision and accuracy, 12 different cards are analyzed. An accuracy of 108% with a precision of 6.4% are obtained. This gives us a blood collection accuracy of ± 15% and a precision lower than the threshold of 15%.

Conclusion/Discussion: New precise blood absorption device for THC analysis gives good precision and accuracy results with appropriate stability results. Combined with a fast LDTD-MS/MS analysis using low sample volumes, THC concentrations can be reported quickly to police enforcement.

Keywords: THC, Blood, LDTD-MS/MS

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Development of a new Homogeneous Enzyme Immunoassay for the Detection of Mitragynine in Human Urine

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Background/Introduction: Kratom, the common name for the plant *Mitragyna speciosa*, is commonly used in Southeast Asia for its opioid agonistic properties. The main alkaloids in Kratom include mitragynine, speciogynine, speciociliatine, paynantheine, and 7-hydroxymitragynine. The common route of ingestion consists of chewing or smoking Kratom leaves as well as drinking tea brewed using Kratom leaves. In the United States, Kratom can be purchased in various forms, including capsules, powders, e-liquid, and chocolate bars. Kratom is not a controlled substance, but is listed as a "drug of concern" by the U.S. Drug Enforcement Administration.

Objective: The objective of this study was to develop a new homogeneous enzyme immunoassay that can detect Mitragynine in urine using the CEDIA™ Technology. Further, the antibody will have minimal cross-reactivity to opiate compounds.

Method: CEDIA Technology is based on the bacterial enzyme b-Galactosidase which has been genetically engineered into two inactive fragments, Enzyme Acceptor (EA) and Enzyme Donor (ED). These fragments spontaneously re-associate to form an active enzyme. In the absence of analyte from the sample, the specific antibody binds the ED-drug conjugate causing a decrease in enzyme activity. The free drug in the sample will compete for the limited number of antibody binding sites, making the ED-drug conjugate available for complementation to form an active enzyme. This phenomenon creates a direct relationship between the drug concentration in urine and enzyme activity. The enzyme activity is then determined spectrophotometrically at 570 nm. The performance of the assay was evaluated on the Beckman Coulter AU680 analyzer.

The Mitragynine assay uses a 50 ng/mL cutoff calibrator with controls at ± 50% of the cutoff. Patient samples were obtained from pain management laboratories. Method comparison and cross-reactivity studies were performed to determine the overall performance of the assay.

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Results: The proof-of-concept studies performed here demonstrate that the selected monoclonal antibody is specific to Mitragynine, does not cross-react with 7-hydroxymitragynine, and has no significant cross-reactivity to opioid compounds, tricyclic antidepressants, synthetic cannabinoids, as well as synthetic cathinones. In addition, a 5-day mini-precision study demonstrated good precision in both qualitative and semi-quantitative mode for the cutoff calibrator and controls (< 2% and < 5% CV in qualitative and semi-quantitative mode, respectively). More than 800 urine samples were tested by immunoassay, and samples confirmed for mitragynine by LC-MS/MS showed ≥ 95% agreement between immunoassay and LC-MS/MS.

Conclusion/Discussion:

The preliminary data on the proof-of-concept CEDIA Mitragynine Assay demonstrates good precision, specificity and sensitivity to mitragynine without any significant cross-reactivity to other commonly abused drugs or novel psychoactive substances.

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

Keywords: Mitragynine, Kratom, Immunoassay (CEDIA)

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Development of a new Homogeneous Enzyme Immunoasay for the detection of Merperidine and its Metabolite Normeperidine in Human Urine

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Background/Introduction: Meperidine, also called Pethidine and sold under the trade name Demerol, is a synthetic opioid used to treat moderate to severe pain. Meperidine is metabolized in human liver by N-demethylation to normeperidine, which causes neurotoxicity and has harmful effects after long term use. Both meperidine and normeperidine may be hydrolyzed to the inactive metabolites meperidinic acid and normeperidinic acid followed by glucuronidation to form meperidinic acid glucuronide and normeperidinic acid glucuronide, respectively. About 7% of parent drug is eliminated unchanged in urine together with about 17% as normeperidine, 42% as meperidinic acid glucuronide and 23% as normeperidinic acid glucuronide. Meperidine has a short half-life of approximately 2-5 hours, while normeperidine has a longer half-life of about 15-30 hours.

Objective: The objective of this study was to develop a new homogeneous enzyme immunoassay that can detect meperidine and its active metabolite normeperidine in urine using the CEDIA™ Technology. Further, the antibody will have minimal cross-reactivity to other opioids and structurally similar compounds.

Methods: CEDIA Technology is based on the bacterial enzyme b-Galactosidase which has been genetically engineered into two inactive fragments, Enzyme Acceptor (EA) and Enzyme Donor (ED). These fragments spontaneously re-associate to form an active enzyme. In the absence of analyte from the sample, the specific antibody binds the ED-drug conjugate causing a decrease in enzyme activity. The free drug in the sample will compete for the limited number of antibody binding sites, making the ED-drug conjugate available for complementation to form an active enzyme. This phenomenon creates a direct relationship between the drug concentration in urine and enzyme activity. The enzyme activity is then determined spectrophotometrically at 570 nm. The performance of the assay was evaluated on the Beckman Coulter AU680 analyzer.

The CEDIA Meperidine Assay uses a 200 ng/mL cutoff calibrator with controls at ± 25% of the cutoff. Patient samples were obtained from pain management laboratories. The samples were analyzed by LC-MS/MS to determine the levels of meperidine and normeperidine. Precision, method comparison and cross-reactivity studies were performed to determine the overall performance of the assay.

Results: Proof-of-concept studies demonstrated the selected monoclonal antibody is specific to meperidine, with approximately 50% cross-reactivity to its metabolite normeperidine. The antibody demonstrated < 1% cross-reactivity to opioids and other structurally related compounds, and < 0.5% cross-reactivity to other structurally unrelated compounds, such as tricyclic antidepressants, benzodiazepines, barbiturates, cannabinoids, and amphetamines. Of the 535 patient urine samples that were tested, 10 samples were positive and 525 samples were negative by immunoassay. All the positive samples and 50 negative samples were confirmed by LC-MS/MS. This result demonstrates the assay has excellent agreement between the immunoassay and LC-MS/MS. Five-day mini-precision studies using the cutoff calibrator, low and high controls demonstrated < 2% CV in qualitative mode and < 5% CV in semi-quantitative mode, for within-run and total-run precision.

Conclusion/Discussion: The preliminary data on the proof-of-concept CEDIA Meperidine Assay demonstrates excellent precision, specificity and sensitivity to meperidine and its metabolite normeperidine, without any significant cross-reactivity to other commonly abused drugs.

NOTE: The assay is currently in development and is not approved by FDA.Keywords: Meperidine, Metabolites, Immunoassay (CEDIA)

POSTER

P47

Evaluation of Simplified Workflow for Hair Matrix Extraction Prior to LC-MS/MS Analysis.

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Background/Introduction: Hair analysis is growing in popularity due to the non-invasive nature of the sample collection. Although not used routinely as other matrices such as blood or urine it does have advantages in that the matrix can indicate prolonged drug exposure. This can provide valuable information with respect to therapeutic drug regimens or in abused drug abstinence cases. Sample preparation for hair analysis is often lengthy involving multiple manual labour steps. This poster aims to demonstrate workflow advantages for hair analysis from matrix homogenization, extraction and analysis.

Objective: The objective was to develop a fast and simple workflow for hair samples, from sample handling to quantitative results, with manual steps removed where possible. Extraction using supported liquid extraction (SLE) using an automation platform prior to UPLC-MS/MS analysis was also the goal. Drug suites investigated included amphetamine type, benzodiazepines and Z-drugs, cocaines, opiates, fentanyls, and buprenorphines.

Method: Hair samples of 10-20 mg were weighed and transferred to 2 mL reinforced tubes prior to the addition of 2.4 mm metal beads. Methanol (1 mL) was pipetted, either with or without pH modification and the samples were subjected to micro-pulverization using the Lysera bead disruption system. The methanolic extracts were applied directly to supported liquid extraction, ISOLUTE® SLE+ in 400 µL capacity 96-well plates or columns. Water-immiscible solvents MTBE, DCM and DCM/IPA (95/5, v/v) were evaluated to achieve partitioning and therefore recovery of the drugs of abuse panel. Manual positive pressure processing was compared to the Extrahera automated sample preparation platform. Extracts were evaporated at 40 °C and reconstituted in mobile phase. LC-MS/MS analysis was performed using a Waters ACQUITY UPLC coupled to a Quattro Premier XE triple quadrupole mass spectrometer.

Results: A panel of widely abused drugs was investigated comprising of: amphetamine type, benzodiazepines and Z-drugs, cocaines, opiates and opioids, fentanyls and buprenorphines. Hair extraction was investigated to determine the maximum matrix/solvent proportions within the Lysera-homogenization portion, and the supported liquid extraction portion of the method. Methanol with or without pH modification was chosen for the extraction solvent due to hair-swelling ability allowing effective analyte release from the matrix prior to sample clean-up using supported liquid extraction. In order to reach SoHT limits of quantitation, we compared methanolic extract evaporation and concentration and subsequent minimal dilution with aqueous solvent, versus direct extraction of the methanol extract. Typical recoveries were greater than 80%, with RSDs below 10% and good matrix factors and overall signal response. A range of extraction solvents were applicable depending on the exact panel required. Calibration curves of 10-1000 pg/mg demonstrated good linearity with r^2 values greater than 0.99 for all analytes. LoQs were below the required SoHT quidelines for analyte panels for both screening and confirmation. Full details of the optimized workflow will be demonstrated.

Conclusion/Discussion: This poster fulfills the objective and demonstrates a simplified workflow for the analysis of a range of drugs of abuse from hair matrix, from sample homogenization, through to extraction, cleanup, evaporation and finally quantitative analysis. Extrahera processing allowed an automated extraction procedure, directly comparable to offline positive pressure processing, which generates time savings for laboratory personnel.

Keywords: SLE (Supported Liquid Extraction), Hair, Drugs of abuse

POSTER

P48

Mixed-mode DPX Tips for Comprehensive Drug Analysis in Oral Fluid

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Background/Introduction: Dispersive pipette extraction (DPX) tips have been commercialized and used for analyzing oral fluid for several years. Abstracts of the use of DPX tips have been presented at SOFT that highlight the advantages they offer over other solid-phase extraction (SPE) products in terms of speed and feasibility with automation. In this research study, improvements in recoveries of drugs from a single comprehensive analysis are presented. In order to achieve the comprehensive recoveries, the sorbent used was mixed-mode that contains both weak anion exchange and strong cation exchange resin.

Objective: The objective of this study is to develop a rapid and automated method for analyzing comprehensive drugs in oral fluid. The use of mixed-mode sorbent is evaluated to provide reproducible and high recoveries of acidic, neutral and basic drugs commonly found in forensic applications.

Method: Both Oral-Eze and Quantisal® buffer solutions with negative saliva (500 µL) were spiked with 50 µL of 1% formic acid and 15 µL of a comprehensive drug standard (in methanol) that includes opiates, opioids, benzodiazepines, barbiturates, THC, antidepressants, amphetamines, and cocaine. Concentrations ranged, depending on the analytes, as low as 0.1 ng/mL to as high as 500 ng/mL. A Hamilton Nimbus-96 was used to perform all extractions of oral fluid. The extraction involved the steps of conditioning with 500 µL 50% methanol in water, aspirating and dispensing the solution 8 times, washing twice with 400 µL of 10% methanol in water, and elution with 400 µL of 78/20/2% ethyl acetate/methanol/ammonium hydroxide. The solutions were subsequently evaporated and reconstituted in 125 µL 10% methanol in water.

All analyses were performed using a Thermo TSQ Vantage triple quadrupole MS system coupled to an Agilent 1260 LC system equipped with an Agilent C18 column (Poroshell EC-C18 2.1 x 50 mm, 2.7 µm).

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Results: All drugs analyzed, in both Oral-Eze® and Quantisal®, by the mixed-mode DPX tips had recoveries that ranged generally from 70% to 100%. It was found that less amount of WAX-CX sorbent was required for Quantisal® than Oral-Eze® to achieve similar recoveries for most of the drugs, which may be useful if considering elution with lower solvent volume. However, higher recoveries were generally obtained for THC when using Oral-Eze®, which we believe is due to a higher content of surfactant. The %C.V. for all of the drugs analyzed in the spiked oral fluid buffers ranged from 3 to 12%.

The DPX extractions took less than 15 minutes to complete (for 96 samples simultaneously). Although some steps could be omitted, such as the conditioning step and reducing some of the aspirating and dispensing steps of the oral fluid samples, we chose to focus on a more robust and reproducible method than speed. Nevertheless, this extraction time is very rapid compared to other SPE methods.

Conclusion/Discussion: This study demonstrates a rapid, efficient and automated method for analyzing comprehensive drugs in oral fluid using mixed-mode DPX tips.

Keywords: Oral fluid, automation, DPX tips

P49

A Preliminary Research Study of the Detection and Analytical Quantitation of Synthetic Fentanyl Analogues in Human Urine & Serum using an Ultivo LC/TQ

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Background/Introduction: Considerable scientific research effort is targeted towards the toxicological analysis of new & emerging powerful synthetic opioids such as the fentanyl types. Demonstrating the ability to analyze such synthetic fentanyl analogues using modern analytical research instrumentation in sufficient concentration ranges, with accuracy, precision and confidence is the aim of this research project.

Objective: This preliminary research study aims to illustrate the proof of principle for a potential sensitive, robust and relatively fast targeted analytical research method for the quantitation of 12 synthetic fentanyl opioids, 4-ANPP the synthetic precursor molecule and a similar powerful opioid-like synthetic known as W-18. Simple sample preparation routines were employed to make samples ready for analysis using an Ultivo triple quadrupole mass spectrometer LC/MS (LC/TQ) from both human serum and urine matrices.

Method: LC/MS analysis was performed using an Agilent 1290 UHPLC/Ultivo LC/TQ with electrospray ionization (ESI) in positive mode. The chromatographic column used was a Poroshell EC C18 column (2.1x50mm, 2.7 µm). The UHPLC mobile phases used were 0.01% formic acid and 5mM ammonium formate in water (Mobile Phase A) and 0.01% formic acid in methanol (Mobile Phase B). The total chromatography cycle time was 7 minutes. Two MRM transitions are monitored for the analyte and a single transition for the deuterated or C^{13} internal standard.

Negative serum matrix (250µL) was spiked with calibrators at various concentration levels, cold acetonitrile (500µL) containing the deuterated internal standard was added to affect protein precipitation and centrifugation was performed at 5000rpm. The supernatent liquid was then further diluted (1:2) with a 10:90 methanol:water solvent mixture prior to instrument injection.

Negative urine was spiked with internal standards and specified calibration levels, centrifuged at 5000rpm (4ºC) for 10 minutes, then 100µL of the supernatant was made up to 1mL in the sample vial by the addition of 900µL de-ionized water.

Results: Excellent linearity and reproducibility were obtained for human serum extracts typically within a concentration range from 10 or 50pg/ml to 500ng/ml (50/250fg on-column to 2500pg on-column) for each synthetic opioid analyte with a linearity coefficient of >0.997 for three batches prepared for this research study. Precision data observed over the three batches resulted with a %RSD variation of < 7% across all calibration levels in this research study.

Typical results for the diluted urine samples yielded an actual concentration range from 50 or 100pg/ml to 500ng/ml (250/500fg on-column to 2500pg on-column) for each synthetic opioid analyte with a linearity coefficient of >0.996 for three batches prepared for this research study. Precision data observed over the three batches resulted with a %RSD variation of < 9% across all calibration levels in this research study.

Generally, it was found that the LLOQ sensitivity for each serum-spiked synthetic fentanyl opioid analyte measured in this research exercise was approximately twice as sensitive of that obtained from the urine-spiked matrix.

Conclusion/Discussion: This preliminary research project demonstrates that the performance of the novel Ultivo LC/TQ with the analytical methodology described herein is producing excellent linearity, precision and sensitivity across the range of 10 or 50pg/ml through 500ng/ml for each respective synthetic opioid in human serum and sensitivity across the range of 50 or 100pg/ml through 500ng/ml for the respective synthetic opioid in human urine.

For Research Use Only. Not for use in diagnostic procedures.

Keywords: Synthetic Fentanyls, Synthetic Opiods, LC/MS

POSTER

P50

Analysis for Alpha-Pyrrolidinovalerophenone and its 2-oxo-PVP Metabolite in Plasma by Liquid Chromatography-Tandem Mass Spectrometry

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Background/Introduction: Alpha-Pyrrolidinovalerophenone (alpha-PVP), a novel psychoactive substance, also known as flakka, gravel, or bath salts, emerged after 2010. Routes of administration include snorting, smoking/inhalation, oral, and rectal. Alpha-PVP has euphoric and CNS stimulation effects. Higher doses and persistent use has the potential to cause severe anxiety, aggressive behavior, and stimulant induced psychosis. Deaths from alpha-PVP have also been reported. 2-oxo-PVP is thought to be a major metabolite of alpha-PVP.

Objective: The widespread recreational use of alpha-PVP and interest in its pharmacological effects has created the need for analytical methods that measure alpha-PVP and its metabolites in biological samples. The goal of this study was to develop a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method that can quantitate alpha-PVP and its metabolite 2-oxo-PVP in rat plasma.

Method: The assay volume was 0.1 mL. The calibrators, controls, and blank were added to separate, clean 16x100 mm glass culture tubes. alpha-PVP-d_8 and 2-oxo-PVP-d_6 were added as 25 µL of 0.1 ng/µL alpha-PVP-d_8/ 2-oxo-PVP-d_6 per tube. Extraction was liquid-liquid with 0.1 mL ammonium hydroxide and 2 mL of 1-chlorobutane:acetonitrile (4:1). Following mixing and centrifugation, the organic layers were transferred into separate, clean 13x100 mm glass culture tubes, and evaporated to dryness under a stream of air using a TurboVap evaporator (Zymark, Hopkinton, MA, USA) set at 40˚C. The extracts were reconstituted with 0.2 ml of 0.1% formic acid and transferred to separate polypropylene 0.3-mL autosampler vials. For LC-MS analysis, an Agilent (Santa Clara CA, USA) 1100 HPLC system coupled with a Thermo Scientific (Waltham, MA, USA) TSQ Quantum Access MS-MS mass spectrometer was used. The LC column was a YMC ODS-AQ, 50 mm x 2 mm, 3 µm column (YMC America, Allentown, PA, USA). The mobile phases consisted of 0.1 % formic acid in water and acetonitrile with a gradient elution (90Ⓡ40:10Ⓡ60) and 0.2 ml/min flow rate. The run time was 18 minutes. Selected reaction monitoring (SRM) was employed in positive ion electrospray and the following transitions were monitored: alpha-PVP: 232Ⓡ91, alpha-PVP-d_8: 240Ⓡ91; 2-oxo-PVP: 246Ⓡ91; 2-oxo-PVP-d_6: 252Ⓡ91. All transitions used a collision energy of 28.

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Results: The chromatographic conditions resulted in the elution of alpha-PVP at 6.4 minutes and 2-oxo-PVP at 8.9 minutes. The calibration range was from 0.25 to 500 ng/mL. A quadratic curve fit and a 1/x weighting was used. For 5 analytical runs, the mean r^2 was 0.996 for alpha-PVP and 0.995 for 2-oxo-PVP. Accuracy and precision were evaluated by analyzing quality control samples prepared at 0.75, 10, and 400 ng/mL. Intra-assay evaluation also included the 0.25 ng/mL lower limit of quantitation prepared in 6 different blank plasma sources. Intra-assay accuracy (n=5) ranged from 88.9 to 117.8% of the target and with intra-assay precision ranging from 0.9 to 16.0% CV. Inter-assay accuracy ranged from 98.7 to 110.7 % of the target with inter-assay precision ranging from 4.5 to 12%. Extraction recovery was at least 52% for alpha-PVP and 67% for 2-oxo-PVP. Matrix effect evaluation showed ionization recoveries of at least 64% for alpha-PVP and 82% for 2-oxo-PVP. Any extraction and ionization losses did not adversely affect the assay performance. Stability evaluation showed that alpha-PVP and 2-oxo-PVP were stable in controls maintained at room temperature for 24 hours. Additionally, they were also stable in processed samples (extracts) stored at room temperature for at least 24 days.

Conclusions: An effective LC-MS-MS procedure for the analysis of alpha-PVP and 2-oxo-PVP in rat plasma was developed and evaluated. This procedure was used to analyze samples from a pharmacokinetic study.

Funding: This work was supported by NIDA contract No. NO1DA-14-7788.

Keywords: Alpha-Pyrrolidinovalerophenone (PVP), LC-MS-MS, Novel Psychoactive Substance

P51

The Incidence And Concentrations Of Fentanyl Found In Post Mortem (PM) Whole Blood Samples Compared To The Incidence And Concentrations Found In Operating Under The Influence (OUI) Samples In The Commonwealth Of Massachusetts

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Background/Introduction: Fentanyl is a potent member of the opioid class of drugs, the analysis this drug in whole blood samples obtained from OUI and PM cases are routinely performed by forensic toxicology laboratories. In this present study, samples of whole blood from both OUI and PM cases are presented. The data from both types of cases was assessed in terms of incidence and concentration.

Objective: This study was initiated with the idea of comparing the incidence and concentrations of fentanyl in PM blood samples with those found in OUI cases in the Commonwealth of Massachusetts after identical SPE and LC MS/MS analysis. The data will assist those analysts involved in the interpretation of fentanyl in both types of cases by providing differential concentrations in each type of case.

Method: Samples of whole blood (0.5 milliliters (mL (Calibrators, controls, and test (PM and OUI) containing 25 ng per mL of deuterated internal standard (fentanyl-d_5))) were diluted with 2.5 mL of phosphate buffer (pH 6), after which the samples were extracted on mixed mode C8/ Strong Cation Exchange SPE columns. The SPE columns were pre-conditioned with methanol, deionized (DI H_20) water and 0.1M phosphate buffer (3 mL, 3 mL, 1 mL respectively) prior to sample loading. The SPE cartridges were washed with DI H_2O, 1.0 M acetic acid, methanol (3 mL, 3 mL, 3 mL), respectively After drying, each SPE column was eluted with 3 mL of a solution of methylene chloride, isopropanol and ammonium hydroxide in a 78:20:2 ratio, respectively. The samples were evaporated to dryness under nitrogen at 40 º Celsius, and the residues dissolved in 100 microliters (µL) of mobile phase (95:5 0.1% formic acid in water: 0.1% formic acid in acetonitrile) for LC-MS/MS analysis was performed in positive multiple reaction monitoring (MRM) mode (details presented). Liquid chromatography was performed in gradient mode with a 5-minute run time.

Results: The limits of detection/quantification for this method were determined to be 0.3 and 0.5 nanograms per milliliter (ng/ mL) respectively. The method was found to be linear from 0.5 ng/ mL to 50 ng/ mL (r^2>0.99) using a weighted 1/x method. Recovery of fentanyl/internal standard were found to be greater than 95%. Interday and Intraday analysis of the compounds were found to < 8% and < 10 %, respectively. Matrix effects were < 6%. The results of OUI cases involving 59 males (mean age= 29 yrs): the mean value was 5.2 ng/ mL (range 0.9 to 12 ng/mL), for OUI cases involving 35 females (mean age 25 yrs): the mean value was 3.8 ng/ mL (range 0.5 to 9.2 ng/ mL). The results of PM cases involving 120 males (mean age= 37 yrs): the mean value was 38 ng/ mL (range 0.5 to 173 ng/ mL), and for PM cases involving 66 females (mean age 32 yrs); the mean value was 29 ng/ mL (range 1.2 to 64 ng/mL).

Conclusion/Discussion: In this project, data was obtained for samples of whole blood submitted for testing in 2017. A total of 1506 PM and 1155 OUI cases were analyzed for fentanyl. This study shows that in the Commonwealth of Massachusetts, fentanyl is observed in PM cases more often than it is in OUI cases. The relative concentrations are greater in males than females in both types of cases. The interpretation of concentrations of fentanyl in PM samples relative to those obtained from OUI subjects should be evaluated based not only on toxicology but case and medical history too.

Keywords: Fentanyl, Toxicology, Analysis

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Cannabinoid Use Pre-Cannabis Legalization in Canada: A Retrospective Review of Forensic Urine Drug Screening Results from 2016 to 2017

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Background/Introduction: New legislation towards the legalization and regulation of cannabis has recently been introduced in Canada. If approved by Parliament, the *Cannabis Act* could become law on July 1, 2018. The impact this Bill may have on cannabinoid use in Canada and workplace safety is unknown. Currently, specific information regarding the relative prevalence of cannabinoid use by the Canadian workforce is not broadly available or published. The use of urine drug screening positivity rates from workers who undergo forensic urine drug testing can be used as a means to obtain this information and evaluate the relative impact of this legalization.

Objective: Identify pre-legalization trends in cannabinoid use by reviewing urine drug screening positivity rates for 11□nor□9□carboxy□Δ9□tetrahydrocannabinol (THCA) from U.S. federally-regulated (U.S. Department of Transportation (DOT)) and non-U.S. federally-regulated (non-DOT) forensic urine drug testing.

Method: Urine drug testing performed in 2016 (N>220,000) and 2017 (N>222,000) by our U.S. Health and Human Services (HHS)-certified forensic drug testing laboratory were retrospectively reviewed. Urine specimens were initially tested using a qualitative DRI™ THC (cannabinoids) drugs of abuse immunoassay screen from Thermo Scientific (positive/negative cut-off 50 ng/mL) on a Beckman Coulter AU5800 automated chemistry analyzer. Quantitative gas chromatography mass spectrometry (GC-MS)-based confirmation testing was performed on all presumptive positive specimens (positive/negative cut-off 15 ng/mL). These specimens were hydrolyzed with potassium hydroxide prior to undergoing liquid-liquid extraction and chemical derivatization with bis(trimethylsilyl)trifluoroacetaminde (BSTFA) with 1% trimethylchlorosilane (TMCS). GC-MS testing using selected ion monitoring (SIM) of the derivatized THCA product was then performed. THCA positivity rates for each donor cohort were tabulated and partitioned by month.

Results: The 2016 THCA positivity rate amongst donors receiving DOT forensic urine drug testing was 0.78%. Although the 2017 positivity rate for this cohort was 0.84% the relative increase from 2016 was not significant ($p>0.05$). The 2016 THCA positivity rate for non-DOT was 6.0% and increased significantly ($p\leq0.05$) to 7.0% in 2017. A trending month-over-month increase in the THCA positivity rate amongst these donors was also observed, with the highest monthly positivity rate being 7.9% in November 2017.

Conclusion/Discussion: THCA positivity rates are significantly different amongst donors whom receive DOT and non-DOT forensic urine drug testing in our Canadian laboratory. The relative prevalence of cannabinoid use in the DOT cohort was consistent in 2016 and 2017. In contrast, cannabinoid use significantly increased in the non-DOT cohort over the same time period. By providing a two-year reference baseline, this study may be used to evaluate the relative impact of cannabis legalization in Canada on cannabinoid use within a specific subset of the Canadian workforce which is subject to DOT and/or non-DOT forensic urine drug testing.

Keywords: Cannabinoid use in Canada, Cannabis Legalization, Urine Drug Screening Positivity Rates

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Fentanyl and Illicit Drug Use Continues to Significantly Increase in Ontario Canada: A Retrospective Review of Urine Drug Screening Positivity Rates from 2014 to 2017

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Background/Introduction: The use of illicit fentanyl and/or of illicit drugs laced with fentanyl presents a heightened overdose risk to the consumer. Their identification is currently reliant upon targeted testing of seized material and/or bodily fluids following an adverse event. Although these potentially lethal products are increasingly being sold in Ontario, Canada specific information regarding the relative prevalence of their use is not yet broadly available or published. It has recently been proposed that urine drug screening positivity rates from a defined population within a specific geographical area may be used as a means to obtain this information and identify significant trends in use.

Objective: Identify the relative prevalence of fentanyl use and its co-ingestion with illicit drugs through a multi-year (2014 to 2017) retrospective review of qualitative liquid chromatography tandem mass spectrometry based (LC-MS/MS) urine drug screening positivity rates from a cohort of subjects tested within a clinical setting in Ontario, Canada.

Method: LC-MS/MS urine drug screening results from 2014 (*N*=136,864), 2015 (*N*=153,329), 2016 (*N*=106,687) and 2017 (*N*=75,774) were retrospectively reviewed. All urine specimens initially underwent a β-glucuronidase enzymatic hydrolysis and protein precipitation sample pretreatment. Prior to injection, each specimen was diluted 20-fold in equal parts aqueous (2 mM ammonium formate with 0.2% formic acid in water) and organic (0.1% (v/v) formic acid in methanol) mobile phases. Screening was performed using a Waters Acquity liquid chromatography system paired with a Waters Xevo TQD triple quadrupole mass spectrometer operated in positive-ion electrospray ionization mode (+ESI). This LC-MS/MS testing protocol screened for a total of *N*=63 different compounds using their respective positive/negative cut-off concentrations. The positivity rates for fentanyl (25 ng/mL cut-off), norfentanyl (25 ng/mL cut-off), amphetamine (250 ng/mL cut-off), methamphetamine (250 ng/mL cut-off), benzoylecgonine (100 ng/mL cut-off), cocaethylene (100 ng/mL cut-off), 6-acetylmorphine (10 ng/mL cut-off), MDMA (250 ng/mL cut-off), and THCA (40 ng/mL cut-off) were tabulated. Urine specimens were considered positive for fentanyl if they screened positive for fentanyl and/or norfentanyl. To identify the prevalence of fentanyl and illicit drug co-ingestion, the relative positivity rates of each illicit drug within all fentanyl positives was also determined.

Results: There was a significant increase ($p \leq 0.05$) from 2014 to 2017 in the positivity rates for fentanyl (6.0% to 6.8%), amphetamine (3.4% to 6.8%), methamphetamine (2.9% to 5.7%), benzoylecgonine (9.6% to 13.3%), cocaethylene (0.4% to 0.6%), 6-acetylmorphine (0.6% to 1.4%), and MDMA (0.06% to 0.1%). The THCA positivity rate (29.6% to 28.6%) significantly decreased from 2014 to 2017. In specimens that screened positive for fentanyl, the positivity rates of several illicit drugs and metabolites significantly increased from 2014 to 2017: amphetamine (4.1% to 13.9%); methamphetamine (4.5% to 15.1%); benzoylecgonine (17.7% to 36.0%); cocaethylene (0.5% to 1.7%); and 6-acetylmorphine (1.5% to 13.3%). With the exception of cocaethylene, the relative positivity rates of these analytes increased significantly year-over-year. The positivity rates did not significantly change from 2014 to 2017 for MDMA (0.06% to 0.15%) and THCA (36.3% to 37.0%) within fentanyl positive specimens.

Conclusion/Discussion: This retrospective review of LC-MS/MS urine drug screening results from 2014 to 2017 identified the relative prevalence of fentanyl and illicit drug use as well as the incidence of their co-ingestion within the studied cohort. The positivity rates of several illicit drugs (cocaine, heroin, amphetamine, and methamphetamine) significantly increased within fentanyl positive specimens over the studied time period. Increased use of illicit fentanyl and/or of illicit drugs laced with fentanyl may be contributing to these significant trends. Urine drug screening positivity rates from a defined population within a specific geographical area can be used as an adjunct approach towards identifying the prevalence of illicit drug use. Communicating trends in urine drug screening positivity rates would serve to educate the broader community about the prevalence and associated risk of using illicit fentanyl and/or fentanyl contaminated street drugs.

Keywords: Illicit Drug Use; Fentanyl; Urine Drug Screening Positivity Rates

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Simultaneous Determination Of Synthetic Cannabinoids And Their Metabolites In Human Hair Using LC-MS/MS And Application To Human Hair

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Background/Introduction: Hair is one of the most important samples for judging the abuse of drugs in the field of forensic science. However, few studies have been conducted on synthetic cannabinoids and their metabolites in human hair. Synthetic cannabinoids are a class of chemicals that bind to cannabinoid receptors but are structurally different from the cannabinoids found in cannabis. They have been sold sprayed on dried, shredded plant material under brand names such as "Spice" since the 2000s. In South Korea, synthetic cannabinoids have been widely distributed since 2009.

Objective: In this study, we developed simultaneous analytical methods for the detection of 18 synthetic cannabinoids and 41 of their metabolites applied to authentic human hair using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Method: Possible contaminants on the surface of hair samples were eliminated by washing with methanol and distilled water, respectively, using reservoirs. Hair samples (about 20 mg) were cut finely into 1 mm pieces with scissors and analytes were extracted by incubating with 2 mL of methanol at 38□ with continuous stirring for 20h. JWH-018-d_9 and JWH-018 N-(5-hydroxypentyl) metabolite-d_5 were used as internal standards for the parent drugs and metabolites, respectively. The extracts were collected in glass tubes and evaporated to dryness under nitrogen gas at 45□. The residues were reconstituted in 100 µL of a 1:1 (v/v) mixture of methanol and mobile phase, and then filtered (0.22 µm, PVDF). Finally, prepared samples were analyzed by LC-MS/MS with electrospray ionization in positive ion mode. The method was validated according to SWGTOX guidelines[1)] and Peters et al.[2)] with few modifications. Selectivity, linearity, limits of detection (LODs), limits of quantification (LOQs), precision, accuracy, matrix effect, recovery and process efficiency were evaluated.

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Results: The LODs and LOQs ranged from 0.1 to 10 pg/10 mg hair and 0.1 to 20 pg/10 mg hair, respectively. Acceptance criteria was achieved within the range of 1-1000, 10-10000 or 20-20000 pg/10 mg depending on the analytes. The method showed acceptable intra- and inter-assay precision (CV, %) and accuracy (bias, %), which were less than 15%, and matrix effect, recovery and process efficiency were conducted and evaluated. Additionally, the distribution of synthetic cannabinoids in the head hair of Korean drug abusers for 2016-2017 was investigated. Hair samples from 39 individuals suspected of synthetic cannabinoids use were provided by the law enforcement agency. The most detected drugs in the head hair of Korean drug abusers were AB-CHMINACA and JWH-210.

Conclusion/Discussion: A quantitative LC-MS/MS method for the simultaneous analysis of synthetic cannabinoids and its metabolites in human hair has been developed and fully validated. This method was successfully applied to authentic human hair samples.

Keywords: Synthetic cannabinoid, Hair analysis, LC-MS/MS

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Simultaneous Determination of Bentazone and Its Metabolites in Postmortem Whole Blood Using Liquid Chromatography-Tandem Mass Spectrometry

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Background/Introduction: Initially registered in 1975, bentazone (3-isopropyl-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide) is a herbicide that has been used under the trade name Bentazone® and Basagran®. Bentazone has been considered moderately toxic upon ingestion and slightly toxic upon dermal absorption in mammals, and WHO has classified bentazone as slightly hazardous. The acute oral toxicity (LD_{50}) is 400 mg/kg in mice, 750 mg/kg in rabbits, and 1100 mg/kg in rats, but its toxicity in humans is not clearly known. Several analytical methods have been reported in the literature to detect and measure bentazone in water, including HPLC, UV/VIS spectroscopy, gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Although, 6-hydroxybentazone and 8-hydroxybentazone are known as phase I metabolites of bentazone, there are very few reports on the analysis of bentazone and its major metabolites in biological samples. Thus, it is necessary to establish and validate an analytical method for the accurate determination of bentazone and its metabolites in biological samples.

Objective: A liquid chromatography-tandem mass spectrometry method with solid-phase extraction (SPE) was developed and validated for the detection and quantitation of bentazone and its two hydroxylated metabolites, 6-hydroxybentazone and 8-hydroxybentazone, in postmortem blood. The validated method was applied to authentic samples from three fatal cases from 2016-2017 for the determination of bentazone and its corresponding metabolites levels.

Method: The blood samples were diluted with 3 mL of 0.1 M phosphate buffer, vortexed for 15 s, and then sonicated for 15 min. After centrifugation at 3000 rpm for 5 min, the supernatant was loaded onto Oasis HLB SPE cartridges preconditioned with 2 mL of methanol and 2 mL of deionized water. After loading, the cartridge was washed with 2 mL of 5% methanol and the analytes were subsequently eluted with 3 mL of methanol. The eluates were evaporated under a stream of nitrogen at 45□. The residues were reconstituted with 300 µL of methanol and then filtered with a 0.22 µm PDVF syringe filter. A 3 µL aliquot was injected into the LC-MS/MS with electrospray ionization in negative ion mode. The mobile phase was composed of 0.1% formic acid in DW and 0.1% formic acid in methanol, and delivered at 0.3 mL/min in gradient mode.

Results: The limit of detection (LOD) of bentazone, 6-hydroxybentazone and 8-hydroxybentazone were 0.05, 0.5, and 0.5 ng/mL, respectively. A good linearity was showed within the range of 5-500 ng/mL in all three analytes and the method showed acceptable intra- and inter-assay precision (CV, %) and accuracy (bias, %), which were less than 15%. The matrix effect values of bentazone, 6-hydroxybentazone and 8-hydroxybentazone were 75.3%, 72.3% and 69.3%, respectively. The recovery values of bentazone, 6-hydroxybentazone and 8-hydroxybentazone were 103.6%, 82.9% and 82.8%, respectively. The process efficiency values of bentazone, 6-hydroxybentazone and 8-hydroxybentazone were 77.9%, 59.9% and 57.4%, respectively. The validated method was applied to authentic samples from three fatal cases from 2016-2017 for the quantitative determination of bentazone and its corresponding metabolites. The concentration ranges of bentazone, 6-hydroxybentazone, and 8-hydroxybentazone in the heart blood from the three victims due to pesticide poisoning were 46.0–91.8, 4.2–6.2, and 0.2–0.6 µg/mL, respectively.

Conclusion/Discussion: The validated method was successfully applied to determine the concentrations of bentazone and its metabolites in postmortem whole blood.

Keywords: Bentazone, 6-hydroxybentazone, 8-hydroxybentazone, LC-MS/MS

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P56

Analysis of Methotrexate in blood samples by LC-MSMS in two cases: Methotrexate intoxication postmortem case and improper administration case to induce unaware abortion during infertility treatment

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Background/Introduction: Methotrexate (MTX) is an antimetabolite agent to treat many different malignancies including acute leukemia, breast cancer, lung cancer, etc. It is usually administered as an intravenous (IV) injection but may also be administered orally or by intramuscular injection. The half-life of MTX is reported to be 6 - 12 hours and 80% of the dose is excreted through the kidneys. MTX's most frequent side effects are hair loss, vomiting, decreased liver function, oral ulcers, anorexia and hematocytopenia. Therefore, continuous monitoring of the drug's blood level is necessary to minimize side effects at large doses**.** An 85-year-old woman died after 14 days of MTX overdose by IV injection due to pancytopenia after hip surgery in a general hospital. The patient's blood samples were collected at three different times at the hospital and were submitted to our laboratory for quantitative analysis of MTX. The second case involved a 35-year-old woman receiving infertility treatment in a hospital clinic and was mistakenly treated with an incorrect embryo transplant, and subsequent abortion without her knowledge following MTX administration.

Objective: We performed the analysis of methotrexate in blood to determine whether MTX was appropriately administered in both cases.

Method: In order to analyze MTX in blood, 0.2 mL methanol and 0.2 mL acetonitrile were added to 0.2 mL of blood for deproteinization and the supernatants were filtered and analyzed by LC-MS/MS with electrospray ionization in positive ion mode. Desipramine-d_3 was used as the internal standard.

Results: In the first case, blood samples from 8, 12 and 13 days after high dose of MTX were analyzed. As a result of the analysis, concentrations of MTX in blood samples were 1.09 mg/L, 0.16 mg/L and 0.02 mg/L, respectively. In the second case, a blood sample collected after 9 days of MTX administration was analyzed. MTX in blood was detected at a concentration below the limit of quantitation.

Conclusion/Discussion: According to the reported paper, the toxic level of MTX was reported to be 4.5 mg/L and 0.45 mg/L after 24 hr and 48 hr after MTX administration, respectively. In the first case, since the level of MTX was 1.09 mg/L in blood 8 days after MTX injection, it could be concluded that the death may have been caused by complications due to high-dose MTX administration. Also, in the second case, we could prove that the hospital injected MTX to the patient to induce unauthorized abortion. MTX was detected in blood much longer than the drug's reported half-life.

Keywords: methotrexate, blood, LC-MSMS

POSTER

P57

LC-MS/MS Development and Validation for the Quantitation of 24 Antipsychotics and Their Metabolites in Urine

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Background/Introduction: Antipsychotic medications are traditionally used to help relieve symptoms improving quality of life for patients with psychosis. They are increasingly being combined with other medications to treat non- psychotic disorders and other mental health conditions. A method was needed to quantify antipsychotics and their metabolites in patient samples, providing healthcare providers with important data to support therapeutic drug monitoring programs.

Objective: This study was designed to develop and validate a fast, production-friendly LC-MS/MS method for analysis of 24 antipsychotics and metabolites in urine. Aspects of method validation will be highlighted, including challenges encountered during the assessments and their resolutions. The key challenge faced during validation was materials compatibility in long-term storage conditions. Strategies were implemented to deduce the most appropriate conditions for working solution storage, which are highlighted in this work.

Method: For each sample, 50 µL of internal standard (5,000 ng/mL for OPC-3373 D8 and 250 ng/mL for all other deuterated analytes) and 50 µL IMCSzyme solutions were added to 400 µL of sample, followed by 1-hour incubation at 65°C. Samples were diluted with 500uL 4% formic acid, loaded onto an Oasis MCX extraction plate, then washed with 500 uL each of 2% formic acid and 30% methanol. Target analytes (in table below) were extracted with 200 uL 5% ammonium hydroxide in methanol and diluted with 2% formic acid in 25% methanol. Sample preparations were performed by Tecan liquid handling systems, and analyzed on a Waters Acquity UPLC with a Sciex 4500QTRAP in positive ion mode. Chromatographic separation was achieved on a Waters BEH Phenyl column with 0.1% formic acid in water (MPA) and acetonitrile (MPB) using a gradient elution with initial conditions of 10% B adjusting to 40% B over 3 min, flushing the column with 100% B for 0.5 min and returning to initial conditions over 1.05 min with a total run time of 4.7 min. This method used a 2.0 µL injection with a 1 mL/min flow rate.

Analyte	LLOQ	ULOQ	Precursor Ion	Product Ion 1	Product Ion 2
Quetiapine	0.5	125	384.2	253.1	221.1
Lurasidone	5	1250	493.3	120.1	166.1
OPC-3373	10	2500	250.1	164.1	122.1
Clozapine	1	100	327.1	270.1	192.1
Olanzapine	2	200	313.2	256.1	198.0
Buspirone	2	200	386.3	222.1	122.0
Perphenazine	2	200	404.2	171.2	143.1
Risperidone	2	200	411.2	191.1	110.0
Thioridazine	2	200	371.2	126.1	98.1
Iloperidone	2	200	427.2	261.1	233.1
N-desmethylclozapine	2	200	313.1	192.1	270.1
9OH-Risperidone	2	200	427.2	110.1	207.2
Fluphenazine	2	200	438.2	171.1	143.1
Chlorpromazine	2	200	319.1	58.0	86.1
Haloperidol	2	200	376.1	95.0	123.0
Molindone	2	200	277.2	70.0	100.1
Loxapine	2	200	328.1	271.1	193.1
Trifluoperazine	5	500	408.2	141.1	70.0
Asenapine	5	500	286.1	229	166.1
Ziprasidone	5	500	413.1	194.1	159.1
Perphenazine Sulfoxide	5	500	420.2	232.0	143.2
Aripiprazole	10	1000	448.1	285.1	176.1

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Thiothixene	10	1000	444.2	98.1	221.0
Pimozide	10	1000	462.2	328.2	109.0

Results: This method was fully validated under FDA-GLP bioanalytical guidelines and transferrable to a high-volume production environment. Automation was assisted with Tecan liquid handling systems, allowing for higher throughput. Materials compatibility and solvent optimization (pH adjustment, organic content and storage material and temperature) was carried out to account for analyte precipitation and non-specific binding encountered during development and validation. Linearity was established with 6 point calibration curves. Precision and accuracy was assessed using four nominal levels, spanning the calibration range, for both intra- and inter-day studies.

Conclusion/Discussion: A production-friendly confirmation LC-MS/MS method was developed and validated for quantification of a range of antipsychotics in urine with a run time of under 5 minutes.

Keywords: Antipsychotics, LC/MS/MS, Method validation

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P58

Identification of Novel Opioid Interferences by HRAM

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Background/Introduction: Novel opioid interferences were observed during the development of a high-resolution mass spectrometry (HRMS) LC-MS/MS urine drug testing method for 47 analytes from multiple drug classes. The interferences affected both analytes and internal standards and were only observed when the method was challenged with patient samples. Some interferences were attributable to isomeric opioid metabolites not previously reported while others were due to interference from in-source dissociations or 13C isotopic contributions from known opioid metabolites not typically monitored as analytes. Based on patient drug profiles, known and inferred metabolism, accurate mass, retention time, and MS/MS spectrum, the putative identity of each interference was assigned and later confirmed, when possible, using an authentic standard. Opioids are some of the most frequently monitored analytes in urine drug testing laboratories. Because of the potential for co-purification, co-chromatography, and spectral similarity, it is anticipated that the reported opioid metabolite interferences could be present with other method conditions and instrument platforms.

Objective: The objectives of this work are to raise awareness of these novel opioid interferences and emphasize the importance of evaluating patient samples for potential interferences during method development.

Method: The primary LC-MS/MS system used for method development studies comprised an Acquity UPLC® I-Class and Q-Exactive™ hybrid quadrupole-Orbitrap™ high resolution accurate mass (HRAM) mass spectrometer). A Poroshell 120 SB-C18 column (2.1x50mm, 2.7-µm particle size) was used for the gradient chromatographic with 0.1% formic acid in water as mobile phase A and 0.1% formic acid in methanol as mobile phase B. TraceFinder™ 3.2 was used for data acquisition and processing. Thermo Xcalibur™ 3.0 was also used for data processing.

The Q-Exactive acquisition method comprised a full-scan (m/z 130-480 at 70,000 resolution) followed by MS/MS scans (17,500 resolution) triggered from a Data-Independent Analysis (DIA) inclusion list. Analyte identity was established relative to a standard by scoring the following qualitative criteria using TraceFinder 3.2: retention time (RT ± 0.15 min), full-scan accurate mass (± 5 ppm window), full-scan isotope pattern (scores range 0-100, and a score of ≥ 70 was used as the positive cutoff), MS/MS accurate mass for at least two expected fragments (± 10 ppm window), and MS/MS spectrum. A custom MS/MS library was built in-house using MS/MS spectra derived from standards. Thermo Library Manager™ 2.0 and NIST08 Mass Spectral Library (user library feature) were used within TraceFinder 3.2 to search the custom library and score the qualitative criteria.

Results: During method development, both internal standard and analyte interferences were observed in the opioid class during the review of patient results.

The D3 internal standards for the 6-keto opioids presented interferences coming from the 13C of the alcohols generated by the reduction of the keto group. Because analyte response is calculated from the analyte-to-internal standard peak area ratio, internal standard interference produces a lower response and potentially false negative results whereas analyte interference produces a higher response and potentially false positive results.

Regarding analytes, the oxycodone metabolites, α/β-oxycodol generated codeine and hydrocodone interferences due to the in-source loss of water. Similarly, the oxycodone metabolites α/β-noroxycodol and α/β-oxymorphol generated norcodeine, norhydrocodone, morphine and norhydromorphone interferences coming from the in-source loss of water. Oxycodone and oxymorphone presented interferences coming from hydrocodone-N-oxide and morphine-N-oxide respectively.

Conclusion/Discussion: Because the interferences were from metabolites not typically monitored as analytes, acceptable proficiency testing results and external control performance are not adequate indicators that a method is free of interference. Methods implemented without being challenged by patient specimens and subsequently scrutinized for interference have the potential for interferences such as those described here. It is important to note that the aim is to raise awareness of the types of interferences that can occur and emphasize the importance of running patient specimens early in the method development process.

Keywords: opioids, interference, high-resolution

POSTER

P59

Drug Screening by LC-QTOF-MS/MS using Protein Precipitation

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Background/Introduction: The Dallas County Southwestern Institute of Forensic Sciences (SWIFS) is a forensic laboratory that does postmortem, human performance, and general toxicology testing. The laboratory's drug screening process was recently updated to a new, highly sensitive LC-QTOF-MS/MS method. The previous method targeted primarily alkaline analytes via a liquid/liquid extraction of a 3mL aliquot of sample and GC/MS analysis. The LC-QTOF-MS/MS method targets alkaline and neutral analytes and their metabolites via protein precipitation, using only 200µL of sample. In addition to the relatively small sample volume, another advantage to this new method is that the data can be retrospectively analyzed if more information about the case becomes available or as the in-house library is updated. SWIFS is currently using the new method for targeted semi-quantitation and qualitative analysis for over 300 compounds with an analytical run time of 12 minutes per sample.

Objective: This poster will serve to introduce members of the community to a sample-conserving, sensitive method that identifies, and in many cases preliminarily quantitates, routinely screened alkaline and neutral drugs and metabolites.

Method: The extraction was performed by adding 200µL of blank blood to the calibrator, positive QC, and negative QC. Each sample (blood, serum/plasma, urine, meconium, liver, gastric, baby formula, milk, or tissue homogenate) was a 200µL aliquot of specimen, and urine dilutions were performed as applicable. 50µL of internal standard were spiked into each tube. 800µL of acetonitrile were added dropwise while vortexing, after which the tubes were centrifuged for 10 minutes at 4400rpm. Supernatants were transferred to 5mL conical tubes and evaporated to dryness using compressed house air and a 55°C water bath. Samples were reconstituted with 200µL of 20% methanol:water and vortexed for 1 minute. Tubes were centrifuged for 10 minutes at 4400rpm and transferred to autosampler vials for injection. The instrumentation used included a Shimadzu Nexera Liquid Chromatography System with a Restek Raptor Biphenyl HPLC column (100x2.1mm, 2.7µm) and a SCIEX $X500_R$ Mass Spectrometer. The ionization source was a Turbo V with Twin Sprayer ESI Probe. Chromatographic separation was achieved using a binary gradient of solvent (A) 0.1% formic acid in water and solvent (B) 0.1% formic acid in methanol at a flow rate of 0.6mL/min. Initial gradient conditions were 5% to 100% B over nine minutes, followed by one minute of 100% B, and finally 5% B for the remaining two minutes. The QTOF was operated in IDA mode using positive electrospray ionization. Fragmentation was achieved using a collision energy of 35V with a spread of ±15V. Data were processed using SCIEX OS Software 1.3 with an extracted ion chromatogram list containing over 300 analytes. An in-house library was created using Certified Reference Materials to establish retention time and fragmentation criteria.

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Results: This method was validated for all semi-quantitative analytes by following SWGTOX guidelines for recommended standard practice for all of the matrices described in the method. Due to the semi-quantitative and qualitative nature of the current method, the range of 0-1000ng/mL was established using a 1-point linear-through-zero calibration curve. Although a single point calibration has higher variability than a multi-point curve, this method will be primarily used for dilution guidance for confirmation assays. Alternative matrices were evaluated at a low concentration to determine if they would meet reporting criteria. Detect-only drugs are repeated for confirmation using the same method, while any drugs needing quantitation are sent to targeted confirmation assays. A positive and negative quality control was included with every batch and all analytes must meet established reportability criteria, including mass error (<10ppm), peak asymmetry (2.5), retention time differences (±0.1 min), signal:noise (10:1), and comparison against the in-house library.

Conclusion/Discussion: This method is currently utilized by SWIFS as part of a routine drug screen. By using this method, analysis time and sample volume decreased, while sensitivity, selectivity, and the scope of analytes identified increased.

Keywords: Drug screening, LC-QTOF-MS/MS, protein precipitation

P60

Inhalants in Impaired Driving

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Background/Introduction: Inhalants are volatile compounds that can cause central nervous system (CNS) depression and related impairment to human performance. Inhalants are infrequently encountered in impaired driving cases and therefore limited studies have been published describing the impact of the use of these compounds on human performance and traffic safety.

Objective: To study and discuss the incidence and impact of inhalants on traffic safety through the presentation of case reports involving these compounds.

Method: From July of 2010 to April of 2018, 2051 blood specimens and 3 urine specimens were analyzed for volatiles for suspected driving under the influence (DUI) cases in Palm Beach County, FL, USA. Specimens were analyzed using headspace gas chromatography with simultaneous flame ionization and mass spectrometry detection. All inhalant positive specimens were also tested for other drugs by scan GC-MS after basic extraction and ELISA, as described previously (1).

Results: Over the study period, ~ 7.8 years, inhalants were identified in 17 cases (0.8%). The time of day and week of the traffic stop or crash is summarized in Table I. Eight subjects were male and nine were female, with a mean (range) age of 32 (19-45) years old. Specimens were collected after a mean (range) of 1.4 (0.4-2.5) hours (29% ≥ 2), and stored at ~4°C for a mean (range) of 15 (2-38) days (29% > 14) prior to analysis.

Toluene was identified in one blood specimen and 1, 1-difluoroethane (DFE) was identified in 13 blood specimens and 3 urine specimens. The incidence of DFE in blood DUI cases was 0.6% during the study period. Other drugs were identified in all but 2 inhalant cases. In the toluene case, methyl ethyl ketone was detected, although not confirmed (not validated at the time of analysis), with no other drugs identified. DFE was most commonly found in combination with benzodiazepines (38%), antihistamines (31%), THC (25%), opioids (19%) and ethanol (13%).

For the toluene case, the officers described the individual as being extremely lethargic and unsteady on his feet with contact cement and nasal discharge on his nose, mouth, chin, neck, shirt and pants. Case histories with a description of the behavior of the subjects were available for 15/16 DFE cases; twelve cases (80%) involved a traffic crash. Signs and symptoms consistent with CNS depressants; such as lethargy (40%), loss of balance (40%), slurred speech (33%), horizontal gaze nystagmus (33%), and confusion (40%) were observed in these cases. Only one case history was available where DFE was the only drug identified in blood. In this case, the driver lost conciousness while driving and crashed. At the scene she demonstrated extreme sedation followed by rapid recovery.

Conclusion/Discussion: DFE was the most commonly identified inhalant over the study period in Palm Beach County, FL, USA, although the analytical method was not capable of identifing nitrous oxide, which has been reported to be a frequently used inhalant. DFE was found in combination with other impairing drugs in the vast majority of DUI cases involving DFE. Inhalant use can cause significant impairment consistent with that produced from other CNS depressants. A high incidence of traffic crashes were observed in cases involving DFE.

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Table I. Time of day and week of the traffic stop or crash (n = 17)

Nighttime	10	59%		Night Weekend	5	29%
Daytime	7	41%		Day Weekend	0	0%
Weekend	5	29%		Night Weekday	5	29%
Weekday	12	71%		Day Weekday	7	41%

Daytime, between 06:00 and 17:59; Nighttime, between 18:00 and 05:59; Weekend, Friday at 18:00 to Monday at 05:59; Weekday Monday at 06:00 to Friday at 17:59

References:

1. Tiscione, N.B., Miller, R., Shan, X., Yeatman, D.T. (2017) Cost/benefit analysis of case management policies in a DUI lab. *Journal of Analytical Toxicology*, **41**, 530-535.**Keywords:** Inhalants, DFE, Impaired Driving

P61

Long-term Phencyclidine (PCP) usage trends in the District of Columbia

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Background/Introduction: Phencyclidine (PCP) was developed in the 1950s as an intravenous anesthetic for both human and veterinarian use under the name SERNYL©. It is known illicitly under a variety of names such as 'Angel Dust'; found in many forms such as powder, crystal, tablet, and liquid; and can be injected, snorted, ingested, or smoked. PCP is a noncompetitive antagonist for NMDA resulting in the high variance in effects observed in users. Other factors such as dosage and chronic use can also contribute to its variability. A comprehensive literature review and a retrospective study on DUI and postmortem cases at the District of Columbia Office of the Chief Medical Examiner (OCME) were conducted in order to demonstrate and draw conclusions regarding trends; reviewing 145 cases from 2003-2017 in which PCP was listed in the cause of death, and 868 driving under the influence (DUI) cases from 2010-2017 in which PCP was present.

Objective: To give background about PCP use specific to the District of Columbia population including key components such as demographics, poly-drug use, and sample concentrations (urine, femoral, heart, and hospital blood). Additionally, the author will illustrate the effects of PCP based on a review of published literature.

Methods: In depth research of medical examiner databases and casework was performed.. A total of 145 OCME postmortem cases (2003-2017) and a total of 868 DUI cases (2010-2017) were reviewed. Data was generated based on key components of the cases such as demographics, poly-drug use, and sample concentrations. Averages were taken per year in reference to age and sample concentrations.

Results: The number of postmortem cases in which PCP was listed increased from 2003-2017; by a factor of 10. Manner of death in the 145 PCP postmortem cases were mostly accidental with only five homicides, three of which were while in police custody. The most common drugs, in order of prevalence, used with PCP in postmortem poly-drug cases were cocaine, ethanol, and heroin. The number of DUI cases in which PCP was present decreased from 2010-2017; by 33.6%. The average age of individuals involved in PCP DUI cases has steadily increased over a 7 year period from 29 to 41-years-old. The most common drugs, in order of prevalence, used with PCP in DUI poly-drug cases were marijuana, benzoylecgonine, and codeine. There is no correlation between femoral, heart, or hospital blood PCP concentrations; they vary, but are all consistent with literature concentrations:

	Postmortem Blood	Antemortem Blood (DUI)
Reference* Average	1.8 mg/L	0.051 mg/L
Reference* Range	1.0-3.3 mg/L	0.012-0.118 mg/L
In-house Average	0.072 mg/L	0.023 mg/L
In-house Range	0.01-0.37 mg/L	0.01-0.15 mg/L

*Reference: Disposition of Toxic Drugs and Chemicals in Man by Randall C. Baselt, 7th edition, 2004.

Conclusion/Discussions: Over the last 15 years the District of Columbia has seen an increase in PCP postmortem cases, most likely contributed by a new administration in 2015 which eliminated inconsistencies with listing specific drugs in cases. Generally, over the last 7 years PCP prevalence in DUI casework has decreased. When comparing OCME casework from 2003-2017, data demonstrates that there is likely no correlation between manner of death and PCP. The fluctuation of PCP use over the last few decades may be due to a phenomenon called "generational forgetting", which is a continuous flow of new drugs onto the scene and of older ones being rediscovered by younger generations.

Keywords: PCP Prevalence, Postmortem and DUI, District of Columbia

POSTER

P62

Method Development and Validation for the Identification and Quantitation of Gamma-Hydroxybutyrate in Urine, Blood, and Oral Fluid Using Gas Chromatography-Mass Spectrometry

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Background/Introduction: Gamma-hydroxybutyrate (GHB) is an endogenous compound in the human body, found in regions of the mammalian brain and believed to be a cerebral neurotransmitter. GHB also acts as a powerful central nervous system depressant commonly used as a "date rape" drug due to its hypnotic and sedative properties. Toxicological analysis of GHB in drug facilitated sexual assault cases is typically performed using urine and/or blood specimens. However, due to the endogenous nature of GHB, toxicological interpretation of these biological specimens can be complex and challenging. Additionally, urine and blood analysis of GHB can be impacted by sample collection, sample analysis times, and sample storage conditions.

Objective: The primary goal of this research was to develop a sample preparation method that could accurately and reliably identify and quantify GHB in oral fluid, an alternative biological matrix. Additionally, this research was carried out to compare the identification and quantitation capabilities of GHB in oral fluid to that of traditional biological matrices, specifically urine and blood.

Method: Urine and blood samples were prepared using 200 uL of matrix, varying amounts of a 200 mg/L working solution, and 50 uL of 100 mg/L working internal standard solution. Liquid-liquid extraction was performed followed by solid phase extraction. Oral fluid samples were prepared using 1.0 uL of drug-free oral fluid, 1.0 mg/mL of GHB (as salt) in methanol, and 1.0 uL of 1.0 mg/mL (as salt) of deuterated internal standard of GHB-d_6 in methanol for quantification. No extraction was required for oral fluid samples. Gas chromatography-mass spectrometry (GC-MS) was utilized for analysis. For each matrix, the following parameters were evaluated: calibration model, bias, precision, limit of detection, limit of quantitation, carryover, and interferences.

Results: GHB and GHB-d_6 could be identified and differentiated due to their fragmentation patterns. All calibration curves for the three matrices exhibited R^2 values > 0.98 using a linear dynamic range of 5 – 100 mg/L with a minimum of four calibration points. The limit of detection for the three matrices was determined to be 1 mg/L, and the limit of quantitation for the three matrices was determined to be 5 mg/L. Bias and precision were analyzed at concentrations of 8 mg/L, 45 mg/L, and 90 mg/L for each matrix. All urine and blood samples were calculated to be within the acceptance range of $\pm$20% bias and coefficient of variation (CV). Oral fluid samples were outside of the $\pm$20% acceptance range for both bias and CV. The highest concentration analyzed that did not produce carryover into subsequent matrix blanks was found to be 350 mg/L for each matrix. Significant interferences were found to be present in urine and blood samples, but negligible for all oral fluid samples.

Conclusion/Discussion: The developed sample preparation method can be used to accurately and reliably identify GHB in oral fluid. Additionally, this research suggests that quantitation capabilities of GHB in oral fluid are not as accurate and precise as those of urine and blood. The developed method has better qualitative analysis capabilities, while the urine and blood methods have better quantitative analysis capabilities for forensic toxicology casework.

Keywords: GHB, oral fluid, DFSA

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Other Drugs present with Positive Suboxone Test Results

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2. Ameritox, LLC, 486 Gallimore Dairy Rd, Greensboro, NC 27409

Background/Introduction: Recent literature has pointed to the coadministration of sedatives (e.g., benzodiazepines) and CNS depressants (e.g., gabapentin) with buprenorphine treatment for opioid addiction (1,2). In some cases, the limit on respiratory toxicity credited to buprenorphine is thought to be removed when these drugs are dosed together (2). Using 6 months of urine drug testing results, the frequency of copharmacy of benzodiazepines including alprazolam and clonazepam, opioids such as oxycodone and hydrocodone, and gabapentin with buprenorphine, both prescribed and not prescribed were examined.

Objective: The data examined herein reflect the coadministration of these sedatives and CNS depressants with buprenorphine for this population. In addition, the presence of a positive test result without the presence of a prescription points to abuse with buprenorphine perhaps to potentiate the effect of benzodiazepines.

Method: These data were obtained from a population of substance abuse treatment programs where testing was used to help monitor patient adherence to their buprenorphine treatment. The methods have been published (3,4) and while the details are not given here, suffice to say these analytes were determined by liquid chromatography mass spectrometry mass spectrometry (LC/MSMS) using transitions reported in earlier work (3,4). Total cycle time was roughly 5.5 minutes using a Phenomenex (Torrance, CA) Kinetex 2.6µm Phenyl-Hexyl 100Å, 50 x 4.6mm (00B-4495-E0). The analyses were carried out by reverse phase chromatography using quadruplexed Thermo Ultra MSMS detectors.

Results: For patients both prescribed and positive for buprenorphine, coadministration of benzodiazepines occurs at frequencies of 3 to 4%. Coadministration of other opioids is very low at less than 0.5% as appropriate and expected. Interestingly, coadministration of marijuana in this group of patients was determined to be 21%, approximately 2x the frequency of use in the normal population (5). Of the other illicit drugs tested, cocaine was present at 5%, as frequent as benzodiazepines! Finally, coadministration of gabapentin occurs in approximately 5% of the samples where gabapentin is prescribed and about 4% when gabapentin is not prescribed.

For patients who are apparently abusing buprenorphine inasmuch as they are positive without a prescription, the rate of coadministration of benzodiazepines is 2.5% whether prescribed or not prescribed with the exception of Xanax™ without prescription which was 7.7%. Interestingly, the frequency of coadministered opioids is 2.3%, almost 5x higher than patients who were prescribed buprenorphine. Administration of gabapentin is higher in this group, approaching 6.5% for those prescribed gabapentin and 5.7% for those not prescribed gabapentin.

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Conclusion/Discussion: For patients prescribed buprenorphine, coadministration of benzodiazepines is present but not a large percentage, either prescribed or not prescribed. For patients not prescribed buprenorphine, coadministration of benzodiazepines can be higher with 7.7% frequency for those not prescribed Xanax™. Interestingly, those prescribed buprenorphine were more likely to be positive for marijuana (e.g., 21%) than those positive for buprenorphine but not prescribed the drug (14.5%); both of which are higher than the normal population in the United States (5). Gabapentin is coadministered between 4 and 6.4% of the time whether prescribed or not prescribed and whether the buprenorphine is prescribed or not. It would not appear that gabapentin coadministration is a large or growing problem in this population.

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Keywords: Suboxone, Buprenorphine, Copharmacy

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A Comparison of Benzoylecgonine Concentrations in Matched Pairs of Umbilical Cord Segments from Twins

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Background: The use of umbilical cord segments for the detection of *in utero* exposure to substances of abuse has been gaining popularity. Multiple births account for 3.5% of all deliveries. One recently published study reported discordant benzoylecgonine results in a matched pair of umbilical cord segments originating from monozygotic twins (Twin A: 0.31 ng/g; Twin B: 5.41 ng/g). To the best of our knowledge, there are no studies evaluating the correlation between benzoylecgonine concentrations in umbilical cord segments from twins. By determining this relationship, we may improve our interpretation of benzoylecgonine concentrations in umbilical cord.

Objective: The purpose of this study was to evaluate the association of benzoylecgonine concentrations in matched umbilical cord segments originating from twins.

Method: This secondary analysis included all umbilical cord drug testing results for specimens received by our laboratory from January 1, 2016 through December 31, 2017 for routine forensic toxicology testing. All specimens were screened by enzyme-linked immunosorbent assay (Cocaine ELISA kit 101315, Neogen, Lexington, KY) and confirmed by liquid chromatography tandem mass spectrometry (Agilent 1200 HPLC System, ABSciex 5500 Tandem Mass Spectrometer) following a solid phase extraction. Benzoylecgonine results were analyzed by the Spearman's rank correlation and Bland-Altman plot to identify the outliers.

Results: Our laboratory received umbilical cord segments from 1503 sets of multiple births. At least one of the newborns was positive for benzoylecgonine in 25 of these cases, of which 21 contained similar benzoylecgonine concentrations in both twins. The concentration of benzoylecgonine ranged from not detected to 888.4 ng/g and were not normally distributed (median 13.4 ng/g; interquartile range (IQR): 1.05, 143.17). Spearman's rank correlation showed a strong positive association of benzoylecgonine concentrations between the two newborns and this observation was statistically significant ($\rho = 0.551$, $p=0.002$). In this study, we discovered four sets of twin specimens received in 2016 and 2017 with discordant benzoylecgonine results (888.4 ng/g, not detected; 11.4 ng/g, 0.7 ng/g; 343 ng/g, 135 ng/g; 426.8ng/g, not detected) as defined by the uncertainty of the confirmation method (26%). Method uncertainty was calculated using the Guide to the Expression of Uncertainty in Measurements (GUM) approach.

Discussion/Conclusion: Based on our observations, most, but not all benzoylecgonine results in umbilical cord originating from twins can be expected to be of comparable concentrations. Limitations of this study include lack of access to the original medical records to verify multiple birth status, and lack of random sampling due to the nature of the study of specimens received at out laboratory. More studies are needed to further explain the discrepancy observed in a few sets of twins.

Keywords: Umbilical Cord, Twins, Benzoylecgonine

POSTER

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GHB Fatalities; Deadly Alone and in Combination

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Background/Introduction: GHB (Gamma-hydroxybutyrate) was previously used as an anesthetic and in the treatment of insomnia and alcohol withdrawal, but is more commonly known as a "date rape" or "club" drug. It is DEA classified as a Schedule I drug – "it has a high potential for abuse, no currently accepted medical use in treatment in the United States, and a lack of accepted safety for use under medical supervision." It is perceived to heighten sexual arousal/desire and euphoria and reduce inhibitions, leading to its popularity in recreational settings. GHB can be addictive and have many serious side effects including hallucinations, dizziness, confusion, nausea, drowsiness, agitation, mental changes, combativeness, memory loss, respiratory depression, seizures, coma, and death.

Objective: The objective of this presentation is to spread awareness of the use of GHB, alone and in combination with other drugs, in suspected overdose cases in which there may not be a thorough or accurate history for the decedent.

Method: GHB and GHB-d6 (internal standard) are drawn out of 200 mcL of sample matrix via liquid-liquid extraction with 250 mcL of 0.1N sulfuric acid and 2 mL of ethyl acetate, rotating the samples for at least 60 minutes. Following transfer of the drug-containing organic layer, samples are evaporated and reconstituted with 70 mcL of ethyl acetate and 30 mcL of BSTFA/1% TMCS (bis-trifluoroacetamide/trimethylchlorosilane) then heated at 70°C for 15 minutes. Samples were analyzed by SIM (selected ion monitoring) Gas Chromatography/Mass Spectrometry. The following ions (*m/z*) were monitored:

GHB-d6 (IS)	239, 120, 206	GHB	233, 117, 204

An initial temperature of 80°C is held for 1 minute, then ramped 20°C/minute until a final temperature of 290°C is reached. The Limit of Detection (LOD) and Limit of Quantification (LOQ) are set as the lowest extracted calibrator of the analysis, 5 mg/L.

Results: Postmortem toxicology results from five (5) cases in which GHB was a contributing factor in the cause of death.

Case 1

A 54-year old male responded to an ad on a social networking site, reportedly drank GHB and engaged in sexual activity, found unresponsive several hours later, pronounced dead on scene.

Cause of death: Gamma-hydroxybutyrate and methamphetamine intoxication.

	GHB	Methamphetamine	Amphetamine	Ethanol
Peripheral blood	310 mg/L	1.3 mg/L	0.10 mg/L	None detected
Urine	Positive	Positive	Positive	**

** testing not performed

Case 2

A 52-year old male with a history of drug abuse went unresponsive/collapsed while meeting a friend,

pronounced dead upon arrival at the hospital.

Cause of death: Multiple drug intoxication.

	GHB	Methamphetamine	Amphetamine	Ethanol
Peripheral blood	12 mg/L	1.0 mg/L	0.08 mg/L	None detected
Urine	None detected	Positive	Positive	**

Case 3

A 27-year old male went unresponsive/collapsed while engaged in sexual activity, pronounced dead

upon arrival at the hospital.

Cause of death: Ruptured cerebral aneurysm.

	GHB	N-Ethylpentylone	Fluorofentanyl	Ethanol

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Peripheral blood	8 mg/L	Positive	Positive	None detected

Case 4

A 36-year old male was found unresponsive during a welfare check, pronounced dead on scene.

Cause of death: Toxic effects of Gamma-hydroxybutyrate.

	GHB	**Fluoxetine**	**THCA**	**Ethanol**
Peripheral blood	195 mg/L	0.11 mg/L	None detected	None detected
Urine	Positive	**	Positive	**

Case 5

A 33-year old male was found unresponsive several hours after sexual activity, pronounced dead on scene.

Cause of death: Acute Gamma-hydroxybutyrate intoxication.

	GHB	**Ethanol**
Peripheral blood	193 mg/L	None detected
Urine	Positive	**

Conclusion/Discussion: Postmortem blood concentrations of 27-1030 mg/L of GHB have been previously associated with overdose fatalities. There are a number of factors that make GHB testing and interpretation difficult including the fact that GHB is naturally occurring in the body, it is rapidly absorbed/metabolized in the body, and no screening test is available, meaning laboratories must rely on case history and/or scene findings to initiate testing. Without a clear association between postmortem GHB levels and toxicity, determination of cause of death relies on the experience of the toxicologist and pathologist and the circumstances of the individual case.

Keywords: Postmortem, GHB, Overdose

P66

Acute Intoxication Of Two Adults After Intake Of Alleged Ecstasy Tablets Containing The Synthetic Cannabinoid ADB-PINACA

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Background/Introduction: Drug abuse is associated with various potential health risks and even experienced drug users are at risk, especially due to varying content of active ingredients or the addition of pharmacologically active adulterants. Several intoxication cases and even deaths have been reported due to particularly high contents of active ingredients (e.g. high MDMA concentration in ecstasy pills) or addition of potent analgesics (e.g. fentanyls to street heroin). The increasing number and easy availability of often highly potent new psychoactive substances (NPS) on the market has aggravated this problem.

Objective: We report a case of a 24 and 22 years old couple in need of intensive medical care after the intake of supposed ecstasy tablets containing unexpected compounds.

Method: Urine and serum samples of both patients were screened by immunoassays, GC-MS and LC-MS. A tablet found in their flat, as well as similar tablets and different powders from the apartment of the drug dealer who sold the drugs, were analyzed qualitatively at the Forensic Science Institute using IR and different MS techniques.

During a subsequent criminal investigation against the drug dealer, including charges of drug trafficking and attempted homicide, additional analyses of the previously mentioned samples were carried out using LC-MS^n and LC-MS/MS techniques in order to assess the potential harm of the ecstasy pills.

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Results: The emergency physician, called by the male intoxicated, found him unconscious but responsive in his apartment. His girlfriend was found comatose/cyanotic with tachycardic arrhythmias and severe generalized seizures requiring immediate intubation.

The tablets found in the patients' home and the dealer's apartment contained different amounts of caffeine, taurine, 2-fluoroamphetamine (2-FA), 5-MAPB, the synthetic hallucinogens diphenidine, and methoxphenidine (MXP), the synthetic cathinones α-PVT, and PV-8, as well as the synthetic cannabinoid ADB-PINACA.

All compounds detected in the tablet were also detected in the serum samples of both patients (with the exception of 5-MAPB), suggesting the intake of one of these tablets, or tablets with a similar composition. 2-FA serum concentrations reached pharmacologically active concentrations of 15 and 67 ng/ml, respectively. Serum concentrations of diphenidine (< 1.0 and 4.1 ng/ml), α-PVT (< 1.0 and 1.7 ng/ml) and PV-8 (< 1.0 ng/ml) were in the low ng/ml range resulting in only minor, if any, physiological effects. ADB-PINACA serum levels reached 6.2 and 30 ng/ml. Factoring in the documented symptoms like vomiting, unconsciousness, respiratory insufficiency and seizures, the highly potent synthetic cannabinoid receptor agonist ADB-PINACA was considered to be the main toxic agent and cause of the potentially life-threatening intoxication.

Documents found at the dealer's residence containing several recipes for different drug compositions suggest that ADB-PINACA was added accidentally, instead of an amphetamine derivative when producing the supposed ecstasy tablets. Both patients were released from hospital with no acute physical impairment after a few days.

Conclusion/Discussion: To our knowledge, this is the first report of an accidental intoxication with the synthetic cannabinoid ADB-PINACA after oral uptake of an alleged ecstasy tablet. This case exemplifies the possible health threats of adulterated drugs of abuse and shall remind physicians and toxicologists to check questionable cases for all types of NPS even if the assumed drug preparation or route of administration would initially rule out a specific compound class.

Keywords: case report, synthetic cannabinoid ADB-PINACA, adulterated ecstasy tablets

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Heading To An Automated Semi-Quantitative LC-Msn-Based Screening Of Substances Relevant To § 24a (2) Of The German Road Traffic Act - Step One: Feasibility Study And Validation

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Background/Introduction: In Germany, a relatively high number of driving under the influence of drugs (DUID) cases is dealing with the question of a suspected violation of § 24a (2) of the German road traffic act (GRTA). This *per se* regulation assumes the driving under the influence - and therefore a traffic offense - if the serum concentration of amphetamine, methamphetamine, 'ecstasy' (MDMA, MDA or MDE), morphine, cocaine (or benzoylecgonine), or THC exceeds certain concentration levels. In the lab, serum samples are usually pre-screened by immunoassays (IA) and positive results are confirmed by quantitative LC-MS/MS analysis since neither the qualitative nor the quantitative information from immunoassays is admissible in court. The great benefit of IAs is the high degree of automation regarding sample preparation and reporting of results. However, tests based on antibodies may lead to false positive results due to cross reactivity issues caused by other compounds or false negative results due to sensitivity.

Objective: The aim of this project was to develop a fast and automated LC-MS^n method for the detection of compounds relevant to § 24a (2) GRTA in serum samples, combining the ease-of-use of IAs with the unambiguous identification power of MS analysis.

Method: The first step of this project covered a general feasibility study including two different ion trap data acquisition modes (AutoMS^n and sMRM) for qualitative and/or quantitative analysis. Sample preparation of 0.5 ml serum was performed using liquid-liquid-extraction (LLE) with chloroform/isopropanol, suitable for all analytes except THC. Analysis was carried out with a 5 minutes gradient elution on an Acclaim C18 column using a Dionex LC-system coupled to an amazon speed ion trap. An in-house generated spectra library of analytes and corresponding isotope-labeled analogs were used for automated compound identification in AutoMSn mode. The MS data was also used to extract ion traces of selected fragments for quantitative data evaluation in sMRM mode.

Results: During this study two data acquisition methods were evaluated and implemented: The Auto-MS^n-mode facilitates fast and easy qualitative analysis with automated result reporting whereas the sMRM-mode offers a quantitative estimate of the analyte concentration, simplifying subsequent confirmation analysis. Both methods have been validated according to the guidelines of the German Society of Toxicological and Forensic Chemistry (GTFCh). Selectivity (n = 10) and LODs (2.5 to 7.5 ng/ml) were verified for both methods. For the sMRM-method matrix effects (85 - 120 %) and recovery (> 60 %, except for benzoylecgonine) - at low and high concentration levels each - as well as repeatability (4 - 20% RSD_R) and accuracy - both at low, medium, and high concentration levels - were evaluated according to validation requirements for quantitative LC-MS analysis. Accuracy requirements (bias < ± 15 %) were not met for all analytes, but considering this a semi-quantitative screening method with subsequent quantitation of positive results, the overall results of the validation study were satisfactory for the intended application. Measurement of 60 serum samples from DUID cases showed that neither the AutoMS^n- nor the sMRM-approach generated false positive or false negative results.

Conclusion/Discussion: Although sample preparation is still carried out manually at this point, the developed LC-MS^n approach may be a suitable replacement for IA testing in DUID cases according to § 24a (2) GRTA.

Keywords: DUID, method development, LC-ion trap-MS

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Postmortem Distribution of Lidocaine in an Infant Death Following Rectal Administration

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Background/Introduction: Lidocaine (xylocaine, lignocaine) is used therapeutically as a local anesthetic and an antiarrhythmic. Lidocaine can be administered intravenously, as a dermal patch, nasal spray, or topical gel. Topical lidocaine preparations are available over the counter in strengths ranging from 2 to 5 % lidocaine. The maximum recommended pediatric dose of lidocaine for topical treatment of hemorrhoids is 4.5 mg/kg. Lidocaine has a high metabolic clearance and is rapidly eliminated after oral administration. Rectal lidocaine administration is less well characterized; limited studies have indicated a reduction in first-pass metabolism and a 70% bioavailability (twice that observed for oral lidocaine).

Case History: The death of a 6-month-old female (17 lbs) was reported to the Office of the Chief Medical Examiner of Maryland. History indicated the decedent experienced chronic constipation and several recent falls from furniture to the floor. The caretaker administered an enema to the decedent to relieve her constipation. According to the caretaker, the enema was administered with a 2 mL disposable pipette which was filled with lukewarm water and dispensed into the infant's rectum three times. The product Passion Anal Desensitizing Lubricant (Active ingredient: 5% lidocaine) was used to facilitate administration of the enema. Shortly after administration of the enema, the decedent's eyes rolled back and she began convulsing and stopped breathing. Rescue breaths were administered and emergency assistance was summoned. Despite resuscitative efforts, the infant was pronounced deceased about 45 minutes later. Lidocaine was not administered during resuscitative procedures. Postmortem toxicology testing was positive for lidocaine; alcohol and other drugs were not detected. Monoethylglycinexylidide and 2,6-xylidine were detected in blood and tissue specimens but not quantitated. Autopsy findings were unremarkable.

Objective: There is limited literature describing rectal administration of lidocaine. This study presents postmortem concentrations of lidocaine from an infant death following rectal administration of an OTC gel containing 5% lidocaine.

Method: Lidocaine was quantitated using the lab's alkaline drug extraction procedure. Briefly, internal standard (Mepivacaine) was added to specimens which were alkalinized and extracted with n-butyl chloride then back extracted into sulfuric acid and finally alkalinized and extracted into methylene chloride. Isopropanol was added and the extract was evaporated to the isopropanol layer which was injected into the GC-NPD for analysis. The method was linear from 0.1 mg/L to 2.0 mg/L. A five-point calibration curve and two control concentrations were included in each batch. Specimens were analyzed at a dilution to produce results within the linear range.

Results:

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Specimen	Lidocaine (mg/L or mg/kg)
Heart Blood	27
Femoral Blood	75
Subclavian Blood	53
Liver	49
Kidney	45
Gastric Contents	0.032 mg total
Passion Lube	44,800

Conclusion/Discussion:

The Passion lube applied to the infant contained 5% lidocaine, the highest strength topical product sold OTC. Just 1mL of this product will exceed the maximum recommended topical dose for a 17 lb infant. This case clearly illustrates the dangers of using an OTC product in a manner for which it was not intended. The high strength topical lidocaine combined with the small size of the infant and high bioavailabity of the rectal route of administration resulted in a fatal outcome that was clearly avoidable. The cause of death was ruled lidocaine intoxication with a manner of homicide.

Keywords: postmortem, topical lidocaine, rectal

P69

Field Performance Of The Approved Instrument Datamaster DMT-C™ Based On Control Tests

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Background/Introduction: The evidential breath alcohol instrument DataMaster DMT-C™ is an approved instrument under Canadian DUI legislation since August 4th, 2007. Starting in 2011, it has progressively replaced the instruments in the field in the Province of Quebec (Canada). Breath test results, in Canada, are expressed in mg of ethanol per 100 mL of blood (using a 2100:1 blood to breath ratio), this is equivalent to mg/210 L of breath. To ascertain the proper calibration of the units, a control test is performed prior to each breath sample analysis using a wet-bath simulator which heats an ethanol standard solution at a temperature of 34.0 ± 0.2°C to produce alcohol vapors at a concentration of 100 mg/100 mL. In Quebec, results of control tests must fall within 95-105 mg/100 mL. One of the most frequently encountered challenge to breath test results is based on the claim that the measurement uncertainty is 10 mg/100 mL. This claim is based on the accepted 20 mg/100 mL maximal difference between sequential breath measurements taken at least 15 minutes apart.

Objective: The reported study was conducted to determine the accuracy, precision and stability of the approved instrument DataMaster DMT-C™ and simulators currently in use in the Province of Quebec.

Method: Data collection was limited to breath test retrograde extrapolation requests received by the Toxicology Department between January 2011 and April 2018, as breath-testing results are not centralized and thus unavailable. A total of 1745 control tests results and wet-bath simulator temperature from 188 different DataMaster DMT-C™ units (of the 203 instruments are currently in use) obtained with three different models of simulators (2100, 12V500 and 12V500RevA from Guth Laboratories Inc.) were collected. The accuracy and precision of this infrared-based instrument and attached simulators, were evaluated based on the determination of the mean and standard deviation. The stability of the instruments employed was evaluated in terms of the measurement disparity within a control set (obtained from 2 consecutive subject breath tests).

Results: The mean value obtained for control tests was 100.1 mg/100 mL with a standard deviation of 1.4 mg/100 mL, yielding an accuracy within 0.1% ($(X_{Calc} - X_T)/X_T*100$). The extended uncertainty (95%, k=1.96) was calculated to be 2.8 mg/100 mL ($U = k * s$). The results obtained with the DataMaster DMT-C™ were stable as 60% (n=575) of control set results were identical while 95% (n=915) had a variation of 1 mg/100 mL or less.

The mean recorded temperature from the simulators used to produce the alcohol vapors was 34.00°C with a standard deviation of 0.01°C and an extended uncertainty (95%, k=1.96) of 0.025°C.

Conclusion/Discussion: The accuracy, precision and stability of the results obtained with the approved instrument DataMaster DMT-C™ and associated simulators all fall within the acceptable 95-105 mg/100 mL range. This study demonstrates that the uncertainty of the DataMaster DMT-C™ at a concentration of 100 mg/100 mL is 3.6x lower than the 10 mg/100 mL sometimes alleged in Court.

Keywords: Alcohol, infrared instrument, uncertainty

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Postmortem Distribution of MDMA and MDA from an Accidental Death due to MDMA Intoxication

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Background/Introduction: A 29-year-old female was found kneeling, face-down on her bedroom floor approximately two hours after her last known communication and pronounced dead on scene roughly 40 minutes later. The scene was unremarkable. Three blister packets of ZzzQuil® (one empty), a bottle of Robitussin®, a pack of oral contraceptives, two prescription bottles (Amoxicillin and Docusate Sodium), and wine/beer were the only reported substances found in the apartment. Approximately 18 hours after the decedent was pronounced dead, a medicolegal autopsy was performed and specimens were submitted for toxicological analysis.

Objective: Evaluate the postmortem distribution of MDMA and MDA in various matrices from a MDMA intoxication death.

Methods: Nine specimens were submitted for toxicological examination. An expanded panel was conducted on the priority sample (Femoral Blood 1) which included volatiles analysis, general drug screening, and confirmatory, quantitative analyses by analyte specific methods. Additionally, electrolyte's testing was conducted by biosensor analysis.

After completion of the expanded panel, all other specimens were analyzed using a confirmatory, quantitative GC/MS method for amines. Amines (including MDMA and MDA) analysis was performed via liquid-liquid extraction (LLE) with n-butyl chloride followed by heptafluorobutyric anhydride (HFBA) derivatization. The samples were reconstituted in ethyl acetate and analyzed using an Agilent© GCMS.

Results: Medicolegal autopsy findings included: pulmonary edema (1,300 grams combined lung weight), zonal necrosis of the liver, approximately 25 mL of green material in the stomach with gastric mucosal hemorrhage, and a contusion on the inner lower lip (a possible indication of agonal seizure activity).

From the expanded panel, seven compounds were reported in Femoral Blood 1. Postmortem concentrations of these compounds, excluding MDMA and MDA, were: cocaine detected, 0.03 mg/L benzoylecgonine, diphenhydramine detected, 0.005 mg/L alprazolam, and naloxone tentative. Furthermore, results from electrolyte's analysis on vitreous humor were:

pH	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	Glu (mg/dL)	VUN (mg/dL)	Creat (mg/dL)
7.104	136.2	13.88	120.2	24	11	0.6

Table 1: Electrolyte Results from Vitreous Humor

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MDMA and MDA postmortem concentrations and ratios for all submitted specimens were as follows:

Specimen	MDMA (mg/L or mg/kg)^	MDA (mg/L or mg/kg)^	MDA:MDMA	Fluid or Tissue MDMA: Femoral Blood 1 MDMA	Fluid or Tissue MDA: Femoral Blood 1 MDA
Femoral Blood 1	16.3	0.31	0.01	1.00	1.00
Femoral Blood 2	15.1	0.26	0.01	0.92	0.83
Heart Blood 1	18.0	0.33	0.01	1.10	1.06
Heart Blood 2	18.1	0.28	0.01	1.11	0.90
Urine	5.89	ND*	--	0.36	--
Vitreous Humor	10.3	0.12	0.01	0.63	0.38
Liver	45.9	1.05	0.02	2.81	3.38
Brain	39.7	0.74	0.01	2.43	2.38
Gastric Content	774	ND*	--	47.4	--

^Fluids were reported in mg/L; tissues were reported in mg/kg
*ND – Not Determined

Table 2: MDMA and MDA Postmortem Concentrations and Ratios by Specimen

Conclusion/Discussions: The official cause and manner of death were ruled as an accident due to MDMA intoxication, although it was recognized that suicide is a distinct possibility. Other drugs detected were not significant in quantity and all electrolyte levels were within normal postmortem limits. The extremely high level of MDMA present in the gastric content suggests the route of ad-

ministration was ingestion. However, no tablets were found in the stomach and the formulation in which the MDMA was ingested – suspected tablet or liquid form – is unknown. MDMA and its active metabolite appear to exhibit similar pharmacokinetics, showing a seemingly consistent ratio with respect to the MDA/MDMA concentrations (0.01 – 0.02) in all specimens. The liver and brain concentrations were substantially higher than the femoral blood. The low MDMA concentration present in the urine suggests death occurred soon after ingestion. The MDMA postmortem concentrations in this case are some of the highest reported. While femoral blood remains the preferred sample, our data suggests that heart blood concentrations are comparable to femoral blood, indicating heart blood may be used when necessary.

Keywords: MDMA, MDA, Postmortem Distribution

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Detection of Novel Psychoactive Substances in Urine using the Randox Evidence Investigator

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Background/Introduction: Novel psychoactive substances (NPS), including bath salts and synthetic opioids, are increasingly available on the illicit drug market. Clinical and forensic drug testing laboratories have been tasked with identifying the presence of the increasing number of NPS (synthetic opioids, e.g., fentanyl derivatives) in the blood and urine specimens of drug users. Confirmation and quantitation of NPS by mass spectrometry (MS) is beyond the capabilities of many laboratories due to resource limitations. Laboratories are thus faced with the challenge of qualitatively identifying NPS using immunoassays without MS testing approaches targeted toward these compounds.

Objective: This study assessed the utility of a novel immunoassay screening platform for identifying NPS in urine specimens.

Method: Urine specimens collected from 34 patients, either presenting to a northern Midwest inner-city emergency department or participants in a pain compliance treatment clinic, with a) clinical suspicion of synthetic opioid use, or b) preliminary positive fentanyl immunoassay screen result that was not confirmed by MS, were analyzed using the RANDOX NPSI (Classic Designer Drugs) and NPSII (Designer Fentanyl and Opioids) immunoassays on the Evidence Investigator platform.

Results: Among 34 samples screened using Randox NPI and NPSII immunoassays due to suspicion of NPS, all were positive for at least one NPS. 17 were positive for classic designer drugs, including PNPI, PNPII, mephedrone, BZP, mescaline, and alpha PVP, while 26 were positive for designer fentanyl and opioids, including furanyl fentanyl, acetyl fentanyl, carfentanil, ocfentanyl, and sufentanil. Cross-reactivity studies are pending.

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Conclusion/Discussion: The Randox Evidence Investigator NPS immunoassay screening system identified numerous presumptive positive drugs that could not be detected using traditional immunoassay screening methods. Incorporating the Randox NPS immunoassay screening methods into our urine drug testing workflows has: a) allowed an economic approach to identify patients suspicious for NPS use that would have otherwise gone undetected and b) supported our clinical suspicion of NPS use prior to MS-based confirmatory testing.

Keywords: Novel psychoactive substances, screening, designer drugs

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A Mechanism Based Forensic Investigation into the Postmortem Redistribution of Morphine

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Background: Postmortem redistribution (PMR) refers to the changes that occur in drug concentration after death. One complication in the interpretation of postmortem blood drug concentration is whether the measured concentration accurately reflects the concentration at death. Postmortem drug concentrations vary depending on the site of sampling as well as characteristics of the drugs themselves. While it was originally thought that the primary source of PMR was diffusion from the cardiac tissues, recent research shows that the redistribution from solid organs (e.g. lungs, liver, and myocardium) is a major contributor. It is important for the field of forensics to continue to research commonly used and/or abused drugs to provide further data into PMR and establish significant reference values. These values are particularly valuable in solving the medicolegal issues since medical examiners and coroners use this information to determine a cause and manner of death. Morphine is often found in the postmortem blood samples of patients treated with or abusing the morphine and/or heroin and its causation in death must be considered. Although PMR patterns of morphine have been established in blood, vitreous humor, brain and other tissues from human autopsy material, these analyses cannot account for many unknown variables (dose, environmental conditions, etc). Hence, it is important to characterize morphine's PMR using direct experiments in a controlled environment where dose is known and external factors are kept to a minimum.

Objective: To evaluate the time course of PMR of morphine and its metabolites in blood and tissues after intravenous administration of morphine to rats.

Methods: Morphine (10 mg/kg) was administered to adult Fisher F344 rats (n=24), including both males and females. Blood was collected at 5 min and 1 h post-injection to determine antemortem distribution, followed by euthanasia by CO_2. Central (heart) and peripheral (femoral vein) blood, along with liver, lung and brain tissues were collected at 0, 8, 16, and 24 hours after euthanasia to determine the postmortem redistribution of morphine and its metabolites. Internal standard (deuterated target analytes except for normorphine) was added to 100 µL of sample followed by the addition of 400 µL of acetonitrile while vortexing. After centrifugation, supernatant was collected, evaporated and reconstituted with 50 µL of mobile phase A and B mixture (95:5). Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile. Using an Agilent 1200 Capillary HPLC, separation of target analytes (morphine, morphine-3-glucuronide (M3G), morphine-6-glucuronide (M6G) and normorphine) was performed on a Restek Raptor Biphenyl 50 x 2.1 mm, 5 µm column. LC runtime was 8.0 min. Data was acquired, processed and reported in Agilent MassHunter software. Comparisons among the groups were performed by using one-way ANOVA, followed by the Tukey's post-hoc analysis for pairwise comparisons, and differences were considered at $p < 0.05$.

Results: Analyte concentrations were averaged across animals (n=6) at each time point and compared. All analytes (when present at detectable concentrations) demonstrated no significant changes in concentrations over postmortem time intervals studied with the exception of morphine in lung tissue ($p=0.003$). In the lung, a unique pattern was observed over the postmortem interval with a decrease during the first 8 h and later 24 h postmortem, but there was no significant change at 16 h postmortem, when compared with zero time (i.e. immediately after euthanasia). Levels of metabolites of morphine, including M3G, M6G and normorphine, did not change over time.

Conclusion: Morphine and its metabolites did not show PMR in any sample matrix tested with the exception of morphine in lung tissue, indicating that these samples would be suitable for quantitation in postmortem toxicology casework within 24 h. PMR does occur in lung tissue for morphine, but not its metabolites. Future studies will focus on the evaluation of environmental conditions that could affect PMR of morphine. This project was supported by Award No. 2016-DN-BX-0002, awarded by the NIJ/OJP/US-DOJ.

Keywords: Morphine, Postmortem Redistribution, Pharmacokinetics

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Identification of Desomorphine in Urine

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Background/Introduction: Desomorphine is a semi-synthetic opioid structurally related to morphine. It is the major component of Krokodil, which is abused intravenously and can cause severe side effects, including skin necrosis. Published case reports indicate that there is often a significant delay between actual drug use and seeking medical treatment for its necrotic side effects. Due to these delays, and the absence of toxicological tests to detect its use, desomorphine is rarely identified in suspected Krokodil users. In this study we describe a sensitive method for the identification of desomorphine in urine.

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

Method: Solid phase extraction (CEREX Polycrom Clin II) and LC/Q-TOF-MS (Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS) equipped with an Agilent Poroshell 120 EC-C18 column (2.1 x 100 mm, 2.7 µm) were used to identify desomorphine. A deuterated internal standard (desomorphine-D3) was employed. A targeted analysis was performed using three transitions from each precursor ion. Fragments were structurally identified and transitions were selected to enhance overall specificity. The procedure was validated in accordance with the Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation. The parameters assessed included analytical recovery, calibration model, carryover, bias, precision, limit of detection, limit of quantitation, matrix effect, and interferences.

Results: Solid phase extraction yielded an extraction efficiency of 90%. Quantitative analysis was achieved using a quadratic, weighted calibration model from 0 to 500 ng/mL. Limits of detection (LOD) and quantitation (LOQ) were determined using three sources of matrix in duplicate over three runs. Both LOD and LOQ were 0.5 ng/mL. Bias and precision were assessed using pooled fortified matrix at 2, 250, and 400 ng/mL in triplicate over five runs. Bias was -4%, 0%, and -1% for 2, 250 and 400 ng/mL, respectively. Inter- and intra-assay precision was 4-7% and 3-5%, respectively (2, 250, and 400 ng/mL). Ion suppression and enhancement for the analyte and internal standard were evaluated at 20 ng/mL (-1%) and 400 ng/mL (-7%) using ten independently sourced urine samples. Interferences were evaluated in terms of matrix, contributions from isotopically labeled internal standards, common drugs, and structurally related compounds. In addition to common drugs, twenty-four opioids and opioid metabolites were included in the interference study. Qualitative interferences were evaluated using negative and positive controls fortified with target analyte (5 ng/mL, 50 ng/mL and 500 ng/mL) in the presence of interferent at a 1-, 10-, or 100-fold increased concentration (500 ng/mL). Carryover was evaluated at 500 ng/mL and none was observed.

Conclusion/Discussion: LC/Q-TOF-MS was used to identify desomorphine in urine following solid phase extraction and assay performance was evaluated in accordance with the SWGTOX Standard Practice for Method Validation.

Keywords: Desomorphine, Validation, LC/Q-TOF-MS

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Prevalence of Fentanyl and Fentanyl Analogues Combined with Heroin and Tramadol in Geographical Areas of the United States

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Background/Introduction: Fentanyl is a synthetic opioid used for pain management and as an anesthetic. When applied in emergency medical or surgical procedures, it is administered intravenously. At home, patients can use the transdermal patch, though intranasal and oral delivery options are also available. Given its extreme potency, fentanyl has been abused since shortly after its introduction in the 1960s. Illicit use has seen an increase in recent years, contributing to the growing opioid epidemic in the United States. Drug seizures reported by the Drug Enforcement Administration (DEA) show that tramadol and/or heroin are commonly found in the same batches as fentanyl.

Objective: The objective of this study was to examine the prevalence of fentanyl and fentanyl analogue use within geographical areas of the United States, and to compare the instances of heroin and/or tramadol detection in biological specimens with the appearance of the same drugs in DEA seizures.

Method: Over the course of the past three years, 2,179 urine specimens from a selection of high-risk civilian populations such as prisoners and parolees were analyzed by LC/MS/MS for the presence of well-established and emerging recreational drugs. The Designer extraction allowed the specimen to be tested for a drug panel containing 84 analytes, including all of the fentanyl and fentanyl-related compounds. The General panel extraction tested for 79 analytes, including 6-monoacetylmorphine (6-MAM) – a metabolite of heroin – and tramadol. When testing first began, only fentanyl, norfentanyl, and acetylfentanyl were included in the testing panel. In June 2016, five fentanyl analogues and 4-ANPP (despropionyl fentanyl), a fentanyl precursor, were added. In September 2017, an additional ten fentanyl analogues were added to the testing panel.

Results: 308 specimens (14%), out of 2,179 specimens tested, were positive for fentanyl or a related compound. Of those, 32 specimens (10.4%) contained tramadol, and 64 specimens (20.1%) contained 6-MAM. The most prevalent fentanyl analogues were acetylfentanyl (19.8%), methoxyacetylfentanyl (11%), cyclopropylfentanyl (5.8%) and furanylfentanyl (4.8%). 4-ANPP was detected in 40.2% of the fentanyl/fentanyl analogue-positive specimens. The specimens came from six distinct geographic areas: New England, Mid-Atlantic, Southeast, Midwest, and Pacific. New England had the highest positive rate, with 92.9% of the specimens from that region testing positive for fentanyl or a related compound, followed closely by the Southeast region (86.3%). The Northwest (13.1%), Midwest (12.4%), and Mid-Atlantic (5.9%) regions had a lower positive rate, with the Pacific region having only one positive specimen (0.3%). New England also had the highest positive rate for 4-ANPP (59.3%), while the Midwest region had the greatest diversity, with 11 different fentanyl analogues detected. The tramadol/heroin results were comparable to the drug seizures reported by the DEA, with the Southeast region showing the highest percentage of tramadol or heroin positives in combination with fentanyl or analogues (52.6%). DEA seizures place the North Central and Mid-Atlantic regions in the middle of the pack, and urinalysis results remain consistent (Mid-Atlantic, 44%, and Midwest, 30.8%).

Conclusion/Discussion: Fentanyl abuse continues to be a significant problem in many parts of the United States. The prevalence of 4-ANPP indicates that potential illicit synthesis of fentanyl is a growing concern as well. The number of fentanyl analogues detected in the past year shows the importance of keeping pace with current drug trends. Tramadol and heroin are commonly detected in conjunction with fentanyl and fentanyl analogues, which could indicate concurrent use or adulteration of the product being purchased, making the abuse of fentanyl even more dangerous.

Keywords: Surveillance, Fentanyl, Trends

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Developing a Salt-Supported Dried Blood Spots Method as Novel Approach for Toxicological Analysis of Illicit Drugs and Metabolites Using Gas Chromatography-Mass Spectrometry

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Background/Introduction: In the last years, the use of dried blood spots (DBS) as an "alternative biological sample" in Forensic Toxicology has increased and been highlighted due to several advantages over conventional blood matrices as liquid whole blood, serum, and plasma. DBS reduces the risk of contamination, can be stored under ambient temperature, and requires less volume of blood sample. However, as the volume of blood is reduced, the toxicological analysis of DBS samples requires instrumental techniques with high sensitivity.

Objective: The aim of this study was proposing a novel approach for DBS preparation using a salt substrate for toxicological analysis of common illicit drugs by GC-MS.

Method: The method proposed is based on mixing an aliquot of 130 µL of defibrinated sheep blood's sample enriched with common illicit drugs and some metabolites (cocaine, benzoylecgonine, amphetamine, methamphetamine, MDA, MDMA, and mephedrone) and a salt followed by pressing using a cylindrical steel holder in a manual press, as to produce a tablet containing the salt-protected blood sample. Different masses of salt and salt types were tested as sodium carbonate, sodium sulfate, sodium chloride, and ammonium chloride. Preparation of the salt-supported DBS sample was performed employing a liquid-liquid extraction (LLE) by mixing the salt-supported DBS with deionized water and ethyl acetate followed by vortex homogenization, orbital stirring, and centrifugation. The organic phase was collected and dried under N_2 flow at 45ºC. The derivatization was performed using the ultrasonic assisted method developed in our laboratory using 20 µL of MSTFA at 30°C for 20 minutes. The analyzes were performed by GC-MS. LLE was optimized using Box-Behnken method of experimental design for testing four parameters, homogenization time, extraction time, solvent volume and addition of salts, at 3-levels each. The optimum conditions were evaluated by Response Surface Methodology and Desirability Function. After optimization of extraction method, selectivity/specificity studies were performed for evaluating interferences from blood sample and from salt substrate and similar compounds interferences.

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Results: For salt supported-DBS method, the best salt substrate was sodium carbonate. The best results of optimization tests are as follows: 1 mL of ethyl acetate extraction solvent, homogenization time of 90 s, extraction time of 15 min and no salt addition. Amphetamine, methamphetamine, MDMA and cocaine can be detected in salt supported-DBS. However, some components of sodium carbonate salt interfered in detecting MDA, mephedrone and benzoylecgonine. Endogenous components from blood matrix did not interfere the detection of analytes.

Conclusion/Discussion: Our pilot study results seem promising and interesting as an alternative for DBS use in toxicological analysis, especially for instrumental techniques which are less sensitive, once our method enables the use of a higher volume of blood. In addition, less sample residue is generated, as salt supported-DBS remains in aqueous phase after extraction and it might be treated. However, the interferences from salt detected in our study must be solved, by using a purification of salt or using other sample preparation methods.

Acknowledgement: This work was supported by Coordenação de Aperfeiçoamento do Ensino Superior (CAPES) (#grant Pró-Forense n° 25/2014)

Keywords: dried blood spots, toxicological analysis, gas chromatography-mass spectrometry

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Halfway to Hydrolysis; Characterizing and Surviving Variability in the Enzymatic Hydrolysis of Phase II Conjugates

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Background/Introduction: The hydrolysis of phase II metabolic conjugates is a fundamental component of urine based toxicology testing as many pharmaceuticals and drugs of abuse are excreted partially or entirely as phase II glucuronide conjugates. Enzymatic hydrolysis of glucuronide conjugates is generally the preferred procedure for releasing measurable analytes. Enzymatic efficiency is typically optimized within a sample of synthetic urine or a drug free urine. During routine testing, similar ideal samples are utilized as discrete quality control samples, intended to represent the hydrolysis efficiency of all samples in a given batch.

The assumption that hydrolytic enzymes will perform optimally in every urine sample despite the wide variation of characteristic elements in natural urine is a questionable. The composition of genuine urine varies dramatically from the idealized composition of synthetic urine. Additionally, natural urine samples differ considerably from each other with respect to creatinine levels, salt concentration, pH, and in their content of the over 3000 small molecules described in urine. All of these differences in urine composition have the potential to alter the efficiency of glucuronidase enzymes, and likely cause a portion of submitted specimens to be reported at inaccurately low concentrations, due to poor hydrolysis efficiency.

Objective: We set to develop a strategy monitoring the hydrolysis efficiency for each independent urine specimen within a currently running laboratory developed test. The procedure was designed to identify poorly hydrolyzed samples which were subsequently selected for further investigation. Selected codeine positive samples were reanalyzed using analyte matched, glucuronide conjugated isotopic internal standard, accounting and correcting for hydrolysis efficiency in each individual sample.

Method: A fully validated LC-MS/MS urine assay targeting approximately 200 analytes was utilized as the test system. The methodology utilizes an optimized and validated hydrolysis procedure incorporating a 60 minute incubation at 50°C with KURA Biotec® β-glucuronidase from Red Abalone followed by methanol precipitation and aqueous dilution. According to typical rules, the hydrolysis is considered acceptable if the final concentration of a prepared glucuronide bound control is within ±20% of expected target concentration.

D_3-tapentadol-glucuronide was added to each sample, calibrator, and control to monitor hydrolysis efficiency within individual samples. Free D_3-tapentadol was monitored in each sample and normalized against an additional internal standard of D_3-protriptyline to control for process efficiency and ionization. Approximately 10,000 samples were evaluated in this manner. Poorly hydrolyzed samples based upon low percentage of released D_3-tapentadol were selected for further evaluation. Repeat analysis confirmed that poor hydrolysis efficiency was related to the nature of samples themselves and not the result of analytical variability.

A subset of poorly hydrolyzing samples also containing codeine (historically challenging to release from its conjugate) were selected for further experimentation. To control for codeine-glucuronide hydrolysis, an internal standard of $^{13}C_4{}^{15}N$-codeine-glucuronide was added to the samples prior to processing. Codeine was then quantified using released $^{13}C_4{}^{15}N$-codeine, inherently compensating for the exact hydrolysis efficiency of codeine-glucuronide within individual samples. These results were compared to traditional quantitation procedures.

Results: Approximately 12% of samples were identified as having poor hydrolysis efficiency deviating from the performance of the passing batch hydrolysis controls. The quantitation of codeine was improved by the use of a glucuronide bound internal standard specifically compensating for the codeine hydrolysis efficiency within individual specimens.

Conclusion/Discussion: Enzymatic hydrolysis is a complex process impacted by sample composition and/or the presence of competing substrates, and naturally occurring potential enzymatic inhibitors. Thus, optimizing and monitoring hydrolysis efficiency in idealized control samples does not adequately reflect the hydrolysis of targeted analytes in all samples. The most accurate quantitation of conjugated analytes can be achieved using specific, isotopically labeled, glucuronide conjugated internal standards. The use of conjugated internal standards inherently corrects for sample variations in hydrolysis efficiency.

Keywords: Hydrolysis, Glucuronide, Opiate

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The Simultaneous Analysis of Twenty-Three Drugs including Amphetamines, Antidepressants, Hallucinogenic, Opioids, Designers, and Anesthetics Compounds by UPLC-MS/MS

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Background/Introduction: In forensic toxicology, analysis of drugs in biological fluids is performed to determine cause of death, drug use, or whether someone was driving under the influence. Determining what analytes are present and the concentration of those compounds in a variety of matrices (e.g., blood, urine, or oral fluid) can be complex. It is therefore necessary to have optimal sample preparation and instrumental conditions. Determining the best approach can be challenging due to the amount of time and resources to perform expansive evaluations of sample preparation, stationary/mobile phases, liquid chromatography (LC) conditions and mass spectrometry (MS) operating parameters.

Objective: This project developed and evaluated the use of Solid Phase Extraction (SPE) and LC-MS/MS to identify and quantify select amphetamines, antidepressants, opioids, anesthetics, designer, and hallucinogenic drugs in human whole blood. These were tested using a Shimadzu Ultra-Fast Liquid Chromatography (UPLC) (Shimadzu, Kyoto, Japan) with a 4000 Q-Trap Electrospray Ionization Tandem Mass Spectrometry (UPLC-ESI MS/MS, SCIEX, Waltham, MA, USA) in positive ionization mode.

Method: Samples, calibration curve, and quality controls (QCs) were prepared for solid phase extraction (SPE) in 200µL of drug-free whole blood (Equitech Enterprises, Kerrville, TX, USA) and analytes were spiked at varying concentrations using certified reference standards (Cerilliant, Round Rock, TX, USA and Cayman Chemical, Ann Arbor, MI, USA). Deuterated internal standards were also spiked into the blood at a concentration of 200 ng/mL. QCs were prepared at 20, 125, 450, and 950 ng/mL. SPE was performed with mixed-mode copolymeric Clean Screen DAU columns (UCT Inc., Levittown, PA, USA). Samples were reconstituted in 400µL of Millipore water (Synergy UV-R, MilliporeSigma, Burlington, MA, USA) containing 0.1% formic acid (Fisher Scientific, Waltham, MA, USA).

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The samples were run by UPLC-ESI MS/MS in positive ionization mode. Separation was achieved using a Kinetex F5 2.6µ 100Å 50 x 3.0 mm column (Phenomenex, Torrance, CA, USA) and a binary gradient of Millipore water and acetonitrile (Fisher Scientific), both containing 0.1% formic acid. Mass Spectrometer settings: curtain gas-25psi, collision gas-high, ionspray voltage-2500, temperature-550°C, ion source gas 1 & 2-45.0, and entrance potential-10. Two product ions, one for quantitation and the other for qualitative assessment, were used for each of the analytes. One product ion was used for deuterated internal standards.

Results: Bias, precision, limit of detection (LOD), limit of quantitation (LOQ), calibration model, carryover, and matrix recovery validation parameters were assessed. All analytes had quadratic fit with 1/x weighting, an LOD of 0.5 ng/mL, LOQs were 0.5, 5 or 10 ng/mL, and no observed carryover. Amphetamines ranged from -18.10 to-11.87% for bias, 0.65 to 12.85% for precision, calibration range of 10-1000 ng/mL, and 89-119% recovery. Antidepressants ranged from -7.80 to 3.88% for bias, 1.16 to 10.44% for precision, calibration range of 10-1000 ng/mL, and 83-109% for recovery. Opioids had a range of -8.19 to 8.3% for bias, 0.24 to 13.46% for precision, calibration range of 10-1000 ng/mL or 0.5-500 ng/mL, and 81-118% recovery. Hallucinogens had a range of -7.17-6.89% for bias, 0.99-13.08% for precision, calibration range of 10-1000 ng/mL or 0.5-1000 ng/mL and 87-110% recovery. Designer drugs ranged from -7.32 to 9.26% for bias, 0.38 to 10.75% for precision, calibration range of 10-1000 ng/mL or 0.5-500 ng/mL, and 88-113% recovery. Anesthetics ranged from-17.85 to 6.08% for bias, 0.91-14.33% for precision, calibration range of 10-1000 ng/mL or 0.5-1000 ng/mL, and 85-116% recovery.

Conclusion/Discussion: A method containing twenty-three drugs including amphetamines, antidepressants, hallucinogens, opioids, designers, and anesthetics was optimized and validated in blood using the Scientific Working Group for Forensic Toxicology () guidelines for method validation. SPE provided clean extract for analysis by positive ESI-UFLC-MS/MS.

Keywords: Solid Phase Extraction (SPE), Forensic Toxicology, Multi-Drug Analysis

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Chasing the Dragon: The Continual Evolution of E-Cigarettes

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Background/Introduction: Currently four generations of electronic cigarettes (e-cigs) are on the market: cig-a-likes, mid-size electronic cigarettes, advanced personal vaporizers, and innovative regulated mods. Traditional e-cigs have a coil through which a cotton or silica wick is threaded, such that an e-liquid moves from the tank to the coil for aerosolization. As each generation progresses, the e-cigs become more powerful and easier to manipulate, enabling the user to more easily inhale drugs other than nicotine, (DOTN). Additionally, the new generations allow for products to be used in the e-cigs such as plant materials, waxes, crystals, and dabs.

Objective: The objective of this research was to monitor the evolution of e-cigs, investigate how they are used or manipulated from the traditional model to consume DOTNs, and evaluate the operational temperature of the devices.

Method: E-cigs from generations two, three, and four were purchased to investigate how the e-cigs operate and their potential for use of DOTNs. The e-cigs were purchased from a variety of online vendors to include: Kanger Aerotank v2, Kayfun Lite Styled Rebuildable Atomizer, Magic Flight Launch Box Portable Vaporizer, Haze Technologies Haze Dual V3, V-ONE X Max, Wulf Mods 510 Dome Kit, Vaped Vubbler Glass Attachment, Source Orb 4, and the Donut Kandy Pen. Coil temperatures were determined for the Wulf Mods 510 Dome Kit, Vaped Vubbler Glass Attachment, Source Orb 4, and the Donut Kandy Pen using a Micro-Epsilon dual IR laser temperature sensor with a range of 100 °C to 1500 °C and Compact Connect software version 1.9.8.6.

Results: The Magic Flight Launch Box Portable Vaporizer and Haze Dual V3 had chambers for the heating of plant based materials. The Haze Dual V3 had two chambers, one for plant based and the other for wax-based materials, enabling the heating of 2 different products at once for a poly-drug experience.

Two of the e-cigs analyzed, the Vaped Vubbler and the Wulf Mods, had a coil wrapped around a ceramic rod, allowing for the vaporization of e-liquid and wax products.

Two of the e-cigs analyzed, the Donut Kandy Pen and the V-ONE X Max, had only a ceramic cup atomizer whereas the Source Orb 4 contains atomizer attachments for a metal cup, a coil wrapped around a ceramic rod, and a coil wrapped around a glass rod. The cup atomizers are designed so that wax products can be used more efficiently.

Temperatures of the e-cigs with ceramic cup atomizers and the coil wrapped around a ceramic rod ranged from 700-900 °C. The atomizers with the coil wrapped around the ceramic rod reached higher temperatures than the cup atomizers. The total average for the cup atomizers was 422.5 °C and the total average for the coil wrapped around the rod was 712.7 °C.

Conclusion/Discussion: Changes in the design of the e-cigs have made it easier for the general public to consume DOTNs. A traditional e-cig wicks the e-liquid from a tank via a fibrous wick that is threaded through a coil. New models have replaced the fibrous wick with ceramic or glass or have replaced the coil system with a cup or chamber. These modifications allow users to more easily vape non-liquid substances or consume less liquid, particularly if it has been adulterated. Additionally, these modified devices do not appear as over drug paraphernalia. The temperatures of the modified devices are significantly lower than in the traditional e-cig, which may impact the efficiency of aerosolization.

Keywords: e-cigarette, drug abuse, vaping

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Detection of Propylene Glycol and Glycerol in Urine as Potential Biomarkers of "Vaping"

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Introduction: Electronic cigarettes (e-cigarettes, e-cigs) are battery powered devices used as an alternative to traditional cigarettes to deliver nicotine and/or flavors in aerosols via inhalation. These devices are becoming increasingly popular, especially with smokers wishing to quit or reduce the habit. A large variety of devices and flavor formulations are currently commercially available. The process of inhaling the vapors (or aerosols) produced by e-cigarettes is known as 'vaping'. While the population of vapers is relatively small compared with conventional cigarette users, this population is projected to grow rapidly, surpassing cigarette sales by 2047.

E-cigarette liquid formulations consist of propylene glycol and/or glycerol, known as e-liquids. These compounds are 'generally recognized as safe' (GRAS) in the food/pharmaceutical industry. E-liquids may also contain flavorants, and active drug(s), vitamins or natural products. Published reports regarding the ratio of propylene glycol to glycerol content in e-liquids ranged from 50:50 to 100 percent of either. E-cigarettes have been added to the Federal Food, Drug and Cosmetic Act by the Federal Drug Administration (FDA) and are now in the process of being regulated; alerts have been issued regarding diethylene glycol contamination of e-cigarettes/e-liquids entering the US market, in particular those from China. Ethylene glycol and diethylene glycol are not authorized as ingredients in pharmaceutical products and food, but are allowed as residuals and can be found as contaminants in various consumer products. Concentrations of 0.1% of diethylene glycol or ethylene glycol in e-liquids are considered acceptable and safe.

Objective: To develop and validate a method to quantitate propylene glycol and glycerol, detect ethylene glycol and diethylene glycol, and to assess potential biomarkers of "vaping" in urine

Method: Propylene glycol, glycerol, ethylene glycol and diethylene glycol analysis was performed using liquid/liquid extraction and derivatized with benzoyl chloride. Corresponding deuterated internal standards were used for all analytes except for diethylene glycol (ethylene glycol D-4). Samples were analyzed on a Waters' TQ-D UPLC-MS/MS. Chromatographic separation was performed on an Acquity UPLC BEH C18 1.7um, 2.1 x100mm, 1.7 µm column. The mobile phases consisted of A: 20mm ammonium formate and B: methanol. The mobile phase ramp started with a 50:50 A:B ratio to 100% B over three minutes and held for 0.5 minutes, then returning to 50:50 A:B. The total runtime was 4.5 mins. The method was validated in accordance with SWGTOX guidelines for linearity, precision and accuracy, stability, interference, carryover and limit of detection studies. The seven point (0.5-100 mg/L) calibration curve and low (3 mg/L), mid (20 mg/L), high (80 mg/L) dilution (200 mg/L) and glycol free controls were prepared in saline. Over 100 urine specimens submitted for drug testing were analyzed for propylene glycol, glycerol, ethylene glycol, diethylene glycol, nicotine, and creatinine.

Results: Calibration curves for all analytes were linear between 0.5mg/mL to 100mg/L, (r^2=0.9990); accuracy was ± 20 with intra- and inter-run precision of <15% CV. Analytes were stable for 48 hours post preparation, 2 freeze-thaw cycles and 3 days at room temperature. No interference was observed from analysis of commercially available DAU and TDM controls. Propylene glycol and glycerol urinary concentration ranges were <0.5 - 68 mg/mg creatinine and <0.5 – 22.6 mg/mg creatinine, respectively.

Discussion/Conclusion: Quantitative analysis of 4 glycols in urine by UPLC-MS/MS was validated. Based on available data, the determined results in this study were consistent with normal urinary glycol concentration ranges. Propylene glycol and/or glycerol urine concentrations may not be effective biomarkers of "vaping".

Acknowledgements: This project was supported in part, by the National Institute of Health (DA033934).

Keywords: vaping, glycols, UPLC-MS/MS

POSTER

P80

Novel Psychoactive Substances in Human Performance Cases: A 7 Year Review.

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Background/Introduction: Novel psychoactive substances (NPS) are psychotropic compounds that are created to mimic the pharmacological effects of illicit drugs of abuse. There are many different types of NPS compounds and they are typically classed based on their chemical structural skeleton and/or the intended pharmacological effect. Common NPS classes include benzodiazepines, cathinones, phenethylamines, synthetic cannabinoids, and synthetic opioids. Various generations of compounds have been observed in most of the NPS classes. As governmental regulatory agencies control or ban specific compounds, structurally similar compounds that are not controlled emerge in the illicit drug market. As these compounds are psychoactive and some reported to be very impairing, it is important for forensic toxicology laboratories to keep up to date on recent drug use trends in order to successfully identify these compounds in human performance toxicological investigations.

Objective: The purpose of this case review was to provide information on NPS prevalence and trends in human performance cases submitted to the University of Miami Toxicology Laboratory (UMTL) since 2011. In addition, selected cases of interest are presented to show the significance of detecting NPS in human performance toxicological investigations.

Method: All of the final toxicology reports for human performance cases submitted to the UMTL since 2011 were reviewed. The presence of NPS in these cases was recorded. Specimens submitted for toxicological analysis included blood and urine. Various analytical techniques were used in order to screen and confirm the presence of NPS in these cases. Analytical instrumentation used included enzyme linked immunoassay (ELISA), gas chromatography mass spectrometry (GC-MS), liquid chromatography tandem mass spectrometry (LC-MS/MS), and liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF-MS). Analytical methods were validated using SWGTOX guidelines and were routinely updated to ensure detection of new NPS compounds.

Results: At least one NPS compound was confirmed and reported in 94 human performance cases submitted to the UMTL since 2011. Driving under the influence (DUI) investigations represented 76.5% of the cases in which the presence of one or more NPS compounds were confirmed. The remaining cases that contained NPS compounds were from drug facilitated sexual assault (DFSA) investigations. The most commonly detected NPS drug classes were the cathinone, synthetic cannabinoids, and synthetic opioid drug classes. Generations of NPS compounds in drug classes were observed in these cases. An example of this is from the cathinone class where methylone was the most confirmed cathinone until 2014, when it was replaced by α-PVP and ethylone. Currently the most commonly detected cathinones are n-ethylpentylone and dibutylone. In 84% of the cases, another psychoactive and/or illicit drug was confirmed alongside the NPS compound(s). Two cases in which only a NPS compound was confirmed demonstrated the importance of identifying these compounds in human performance toxicology cases. In addition, the drug paraphernalia found at the scene of both incidents proved to be valuable in confirming the NPS in the human specimens.

Conclusion/Discussion: While the overall prevalence of NPS compounds was lower when compared to traditional drugs of abuse in human performance toxicology cases, it was still beneficial to identify these compounds as finding a NPS can help explain an impairment observed in such cases. Updating methods and obtaining new drug standards can be challenging for laboratories as resources may be limited. As demonstrated by the two case examples, analyzing drug paraphernalia can help direct the toxicological analysis in successfully identifying NPS in human specimens. If the NPS trends continue, new compounds will appear on the drug market and replace the currently observed compounds. Toxicology laboratories need to be proactive in order to successfully identify these compounds in human performance cases.

Keywords: Novel Psychoactive Substances, Human Performance Toxicology, Drug Trends

P81

Cyclopropylfentanyl Exposures in Unsuspecting Heroin and Cocaine Users in Southeast Michigan- Differentiation of cyclopropylfentanyl from methacrylfentanyl and crotonylfentanyl

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Background: Unequivocal identification of cyclopropylfentanyl, first reported in the US and Europe as a cause of death in June 2017, is challenging because its isobars (methacrylfentanyl, crotonylfentanyl) produce identical electron impact (EI) and electrospray ionization (ESI) spectra.

This presentation will describe how to identify a novel fentanyl analog using EI and ESI spectral patterns of known "fentanyls" on GCMS and liquid chromatography ion-mobility-quadrupole time-of-flight (LC-IM-QTOF-MSe. The details of the LC-IM-QTOF-MSe method used for analysis will also be described.

Objective: Cyclopropylfentanyl, methacrylfentanyl and crotonylfentanyl were differentiated using GCMS and LC-IM-QTOF-MS^{E}. We developed a process to identify novel designer fentanyls as exemplified using first two cases of cyclopropylfentanyl exposures from our laboratory.

Methods: 34-year-old female (Case#1) and 27-year-old female (Case #2) with a history of heroin and cocaine use respectively presented (on different days) to the ED for opioid withdrawal (case#1) and chronic drug use related hand infection (case#2). They admitted to heroin (Case#1) or cocaine (Case#2) use and no known fentanyl use.

The urine samples (case#1 and case#2) were subject to immunoassay screening (Roche Diagnostics) and qualitative confirmation by GCMS (EI) and LC-IM-QTOF-MS^{E} (ESI). Sample preparation for GCMS analysis involved liquid-liquid extraction (LLE) and we optimized and validated a salting-out assisted liquid-liquid extraction (SALLE) and a LC-IM-QTOF-MS^{E} method.

Known EI-GCMS spectra fentanyl scaffold were used to interpret the m/z values in both samples and identify the potential fentanyl analog which was then confirmed by LC-IM-QTOF-MS/MS^{E}. We developed a LC method with a slower gradient and separated cyclopropylfentanyl, crotonylfentanyl and methacrylfentanyl..

Results: Patients were discharged without complications. Opiate screen was positive in case#1 and negative in case#2. Prior to this case, our GC-MS compound library had no reference spectra for cyclopropylfentanyl and norcyclopropylfentanyl. GCMS of the urine from both samples revealed an unidentified compound (9.2 minutes). We hypothesized that this compound was "a norfentanyl metabolite" with unfragmented molecular ion ([M]) m/z=244. Fentanyl undergoes loss of 104Da when metabolized to norfentanyl (Mol. Wt.=232). We hypothesized that if the compound at 9.2 minutes was indeed a fentanyl-analog metabolite, then the precursor designer fentanyl would have [M]=348Da (244Da+104Da). Further, the most intense peak in fentanyl GCMS was m/z=245 (loss of 91Da from fentanyl). So, the expected [M] in the GCMS of the suspect designer would be 257Da. The extracted ion chromatogram from the patient sample GCMS at m/z=257Da revealed a compound at 12.1 minutes with GC-MS spectrum resembling a designer fentanyl analog (m/z=257, 189, 146, 109). The substituents on the fentanyl backbone were predicted as methacryl-, crotonyl- or cyclopropyl-. We used reference material for GCMS and LC-IM-QTOF-MS^{E} (m/z=349.2274 ($[M+H]^{+1}$), 188.1428, 105.0692, 228.1377) analysis for unequivocal identification of cyclopropylfentanyl. Use of a LC method with a slow gradient separated the three isobars. The collision cross section (CCS, Å^{2}) obtained from LC-IM-QTOF-MS^{E} analysis could differentiate methacryl-from crotonyl- or cyclopropyl fentanyl. ESI ion-response ratios (349.2274/228.1377) were significantly different ($p<0.001$) for methacryl- vs crotonyl- or crotonyl- vs cyclopropylfentanyl.

Conclusion/Discussion: We describe two cases in which we detected a fentanyl analog not seen in our laboratory before. We used EI-GCMS behavior of the fentanyl scaffold and ESI-LC-IM-QTOF-MS^{E} to separate isobaric compounds crotonylfentanyl and methacrylfentanyl to unequivocally identify cyclopropylfentanyl. Since all three compounds have identical EI and ESI spectra, chromatographic separation is necessary for forensic analysis particularly when cyclopropylfentanyl exposure is a potential cause of death. IM and CCS are useful to help differentiate isobaric designer fentanyls.

Keywords: cyclopropylfentanyl, crotonylfentanyl, methacrylfentanyl

POSTER

P82

Multi-assay ELISA Validation to SWGTOX Standards

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Background/Introduction: Method validation is an essential component of a quality assurance program. The 2013 Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation specifies that immunoassay validations assess limit of detection and precision at the decision point by analyzing three concentrations: no more than -50% of decision point (-50%), decision point, and no more than +50% of the decision point (+50%), in triplicate over three days. To be acceptable, the %CV of each concentration shall not exceed 20% and the mean ± 2SD of each concentration shall not overlap. Since the publication of SWGTOX Standard Practices in 2013, there have been few published reports of ELISA validation results. Recently, the feasibility of the precision requirement, particularly for low concentration analytes, has been questioned.

Objective: To compare ELISA validation results for cocaine metabolite, opiates, oxycodone/oxymorphone, cannabinoids, fentanyl, carisoprodol, benzodiazepines, methamphetamine, amphetamine, phencyclidine, methadone, acetaminophen and salicylate in blood, serum, brain, liver, muscle, spleen and urine to SWGTOX standards.

Method: During method development, concentration-absorbance curves were established to identify decision points that would yield sufficient separation between the negative, -50%, decision point, and +50%, while being appropriate for postmortem and human performance casework. Precision at the decision point was assessed on at least three days by running triplicate samples at -50%, decision point, and +50%. ELISA kits from Immunalysis were used for all target analytes. The ELISA procedure was completely automated using a Tecan Freedom EVO75 robot, Hydroflex washer and Sunrise reader.

Results: Most analytes achieved precision <20% CV at all three concentrations in blood, serum, urine, liver and brain. Muscle and spleen demonstrated greater variability, often exceeding 20% CV for most analytes and concentrations. When mean ± 2SD ranges were determined, nearly all analytes had one or more range overlap, and thus "fail" validation criteria. This finding was consistent across matrices. If intra-day precision was considered, rather than inter-day, a different pattern was observed. Within a given day, precision was frequently acceptable, and the ranges were less likely to overlap.

Conclusion/Discussion: Our extensive validation involving multiple drug targets in several matrices consistently did not achieve the proscribed SWGTOX validation target for the inter-day mean ± 2SD ranges to not overlap. It is not clear if our results are the exception or the norm, as we are aware of only one peer-reviewed report demonstrating successful validation of an ELISA assay to SWGTOX standard practices. We have anecdotal reports of several other laboratories failing to meet SWGTOX standards in their ELISA validations. Our findings and those of others support a need for evidence-based acceptance criteria for ELISA validation. ELISA is inherently variable, and casework is compared to contemporaneously analyzed controls, rather than historical control performance. Intra-day evaluations may be more practical and more reflective of assay performance than inter-day evaluations, as demonstrated in our data.

Keywords: ELISA, method validation, SWGTOX

P83

Improving the Detection of Heroin Use by Using Papaverine Metabolites as Biomarkers.

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Background/Introduction: Opioid usage in the United States has increased over the past decade with prescriptions increasing from 76 million in 1991 to 207 million in 2013. While new regulations have curbed the number of prescriptions written recently, this decrease in prescriptions has resulted in an increase in individuals turning to heroin as a replacement. Heroin-related overdoses have quadrupled between 2000 and 2015.

Most laboratories are analyzing for 6-acetyl morphine (6-AM) and morphine to determine heroin use. This is ideal in a post-mortem setting, but not in most other settings. 6-AM has reported detection window in urine of 2 - 8 hours. Morphine while detectable for several days, its presence may be explained by prescription use. Thus, 6-AM is detectable less than 1 day, and its presence only indicates very recent heroin use.

Alternative biomarkers, noscapine and papaverine, have been proposed. Noscapine and papaverine have reported detection windows of ~24 hours and 1–2 days, respectively. Papaverine metabolites have reported detection windows of up to three days. Noscapine and papaverine are present in opium and may be detected in illicit heroin- depending on the method used for heroin synthesis and geographical origin. In many countries, noscapine is an approved medication, however, neither drug is approved in the United States.

Of the twelve potential papaverine metabolites, only two are commercially available, 6-desmethyl papaverine (6-DMP) and 4', 6-didesmethyl papaverine (4,6-DDMP). No isotopically labeled metabolites are commercially available. Published methods for the detection of papaverine involve gas chromatography mass spectrometry. These methods only involve qualitative identification and none involve quantitative results. No method involves the use of ultra-high-performance liquid chromatography tandem mass spectrometry (UPLC-MSMS).

Objective: To develop a sample preparation method and a UPLC-MSMS method for the determination of two heroin biomarkers, 6-DMP and 4,6-DDMP in urine.

Method: Several sample preparation techniques for the determination of opiates in urine were evaluated, (i.e. liquid-liquid extractions, solid phase extractions (SPE), supported liquid extraction (SLE)). UPLC-MSMS methods for opiates were also evaluated. All analyses were performed on a Waters' TCS-micro LCMSMS system using MRM. Isotopically labeled opiates were evaluated as potential internal standards. Once an acceptable sample preparation technique was determined, the method validation followed SWGTOX guidelines. This included accuracy, interferences, linearity, precision, and stability. Genuine urine specimens containing and devoid of 6-AM were analyzed.

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Results: The Waters' MCX uElution columns were determined to have minimal matrix effect and 78 – 125 % recovery and processes efficiencies. The isotopically labeled opiates, hydrocodone-d6, oxycodone-d6, and 6-AM-d6, were found to have similar results. It was determined that methanol caused the compounds to not be retained on the SPE columns. Methanol present in the sample preparation had to be kept to a minimum, and methanol was removed as a wash reagent. A Waters' BEH C18 column was used for separation with a 20 mM ammonium formate water: 20 mM ammonium formate methanol gradient. Calibration curves were linear from 100 – 50,000 pg/mL. No interferences were observed from analysis of multicomponent -therapeutic drug or drugs of abuse control materials; intra and inter-run precision tests were acceptable. All analytes were stable under refrigerator and freezer storage conditions. From the analysis of 249 urine specimens (82 6-AM positive, 61 suspected heroin cases, and 106 presumed negatives) 6-DMP concentrations ranged from 0 – 1,025,724 pg/mL and 4,6-DDMP concentrations ranged from 0 – 328,580 pg/mL. Neither metabolite was detected in morphine negative specimens.

Conclusion/Discussion: The modified SPE sample preparation technique was rugged and robust for the analysis of the papaverine metabolites, 6-DMP and 4,6-DDMP. The method has sufficient sensitivity to detect the use of heroin beyond the detection window of 6-AM.

Acknowledgements: This project was funded in part by the National Institute of Justice, Research and Development in Forensic Science for Criminal Justice Purposes, 2016-DN-BX-0148, the National Institute of Health (P30DA03393), and the Virginia Commonwealth University Undergraduate Research Opportunities Program.

Keywords: Heroin, Papaverine, Biomarkers

P84

Enzyme Based Electrochemical Biosensor for the Rapid Detection of Opioids in Oral Fluid with Confirmatory Analysis by LC-QQQ-MS

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Background/Introduction: Opioid abuse is one of the most significant drug threats facing the United States today due to the growing number of novel psychoactive substances (NPS) such as fentanyl-analogs. Due to limitations in the field, a rapid, sensitive, and selective screening technique is needed for opioids and opioid NPS. Electrochemistry provides a versatile platform that is sensitive, selective, portable and a low-cost alternative that can be modified using enzymes capable of binding to these opioids and opioid-related compounds. Cytochrome P450s represent a class of enzyme suited for this work due to their role in the metabolism of many xenobiotics.

Objective: This study aims to develop an electrochemical sensing method that provides qualitative and quantitative information about opioids. This method will enhance the forensic toxicology and seized drug workflow in laboratories to speed up screening, optimize analysis and reduce cost and backlogs. Further, it will allow on-site testing of unknown substances by crime scene investigators and law enforcement and provide for non-invasive roadside testing of DUID suspects. Two model drugs were chosen for this pilot study: codeine and fentanyl.

Method: Screen-printed carbon electrodes with cytochrome P450s were used for the redox process of the analytes in solution. The P450 enzyme was immobilized onto the surface of a disposable electrode via a covalent attachment with a self-assembled monolayer (SAM) technique. Cyclic voltammetry was used to create the monolayers and impart functional groups that support the covalent attachment of the P450 enzyme. Chronoamperometry was used to characterize the behavior of the opioids in phosphate buffered saline to generate standard curves from successive additions and to determine the limits of detection. Additionally, a confirmatory method using an LC-QQQ-MS system using dynamic multiple reaction monitoring methods was optimized to include the two model drugs as part of a more extensive suite of pain management drugs for a total of 20 drugs. A solid phase extraction protocol (SPE) was developed to include the model drugs by spiking them into the oral fluid and further LC/MS characterization and quantification. Comparison of results between the electrochemical sensor and LC-QQQ-MS were performed.

Results: The cytochrome P450 enzymes were successfully immobilized on the electrode surface, and the modification of the electrode was confirmed by the cyclic voltammograms produced from the functionalization. Codeine was characterized, and the limit of detection was determined in phosphate buffer at low parts per million levels, 1000 ppb. Similar ranges of detection were obtained for fentanyl in the phosphate buffer solution, 1000 ppb. A panel of drugs, including codeine and fentanyl, was analyzed through LC-QQQ-MS to provide a confirmatory analysis technique for use with this and future studies. After optimization, chromatographic separation of a panel of drugs was achieved, and a dMRM method was developed for the group of 17 opioids, one hallucinogen, and two stimulants. Dynamic MRM was utilized for improved sensitivity and detection capability. Fentanyl was characterized in a similar manner as codeine using cyclic voltammetry and chronoamperometry techniques.

Conclusion/Discussion: Enzyme-mediated redox of opioids was achieved, demonstrating the viability of this approach to their low detection concentrations. Covalent attachment of the enzyme to the electrode allowed for direct electron transfer, improving the sensitivity of the technique. Chronoamperometry demonstrated a linearrelationship between the concentration of the analyte in solution and the current produced. In addition, the suite of 20 drugs showed chromatographic separation with no isobaric interference or problems with co-eluting compounds.

Keywords: Electrochemistry, Biosensor, Opioids

POSTER

P85

Dart-Ms/Ms and Lc-Ms/Ms Comparison for the Analysis of Stimulants in 108 Seized Dietary Supplements

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Background/Introduction: Dietary supplements are very popular worldwide, especially among athletes, and consumption has continually increased in recent decades. The stimulant 1,3-dimethylamylamine (DMAA) has been added to dietary supplements since 2006, and its use has been linked to adverse effects, including deaths, and sports doping cases, which has led to its prohibition in 2010 by the World Anti-Doping Agency (WADA), among others. However, DMAA is still prevalent in dietary supplements, and it's among the most consumed stimulants by athletes, according to data published by WADA.

Objective: Develop screening and confirmatory methods to detect DMAA, synephrine, ephedrine, caffeine, sibutramine, and methylphenidate, in 108 samples of dietary supplements seized by the Brazilian Federal Police (BFP).

Method: Analyses were performed using a Direct Analysis in Real Time ion sources coupled with a Thermo Finnigan TSQ Quantum triple quadrupole mass spectrometer (DART-MS/MS). The samples were dissolved in a water:methanol (80:20) solution and a 2 μL aliquot was dried onto a pyrex glass capillary for introduction to the DART system. A confirmatory method using a Shimadzu Liquid Chromatograph LC-20AD coupled to an Applied Biosystems MDS Sciex 3200 QTRAP (LC-QQQ-MS) was also developed. Ephedrine-D3 and Methylphenidate-D9 were used as internal standards for LC-MS/MS analysis. Chromatographic separations were achieved using a Luna Omega 3 Polar C18 50 x 2.1 mm column with a gradient elution of methanol with formic acid 0.1 %, with a total run time of 8 minutes. The seized samples were analyzed after extraction using an offline solid phase extraction (SPE) method using Bond Elut Certify cartridges. Liquid-liquid extractions were also tested and compared to the SPE method. Liquid-liquid extractions were analyzed on an Agilent 6470 Triple Quad LC-MS/MS system. Chromatographic separation was achieved on a Zorbex Eclipse Plus C18 RRHD 3.0x50 mm, 1.8 μm column with 0.1% formic acid and 5 mM ammonium formate in water (mobile phase A) and 0.1% formic acid in methanol.

Results: For the screening method by DART-MS/MS, all the samples showed at least one stimulant present. DMAA was present in 20% of the samples and always with at least one of the other substances. Caffeine was positive in 98% of the samples, followed by synephrine (46%), sibutramine (44%), ephedrine (39%) and methylphenidate (10%). For the LC-MS/MS method, after the extraction by SPE only three of the six stimulants were detected. DMAA, synephrine, and caffeine showed positive results in 18.5%, 39% and 99% of the samples, respectively, indicating a good correlation with the results obtained by DART. However, methylphenidate, sibutramine, and ephedrine were not initially confirmed with the LC/MS system used. To increase sensitivity a larger sample aliquot was extracted with a different ethanol/ammonium hydroxide extraction protocol in combination with an Agilent 6470 LC/MS which demonstrated higher sensitivity to these compounds. The combination of an extraction protocol using methanol and ammonium hydroxide—along with higher sensitivity gained with the electrospray source—allowed the detection of trace amounts of these drugs present in the supplements. Thus, confirmed results for sibutramine, ephedrine, and methylphenidate in 23%, 11% and 2% of the samples, were obtained respectively.

Conclusion/Discussion: Considering the results obtained for both methods, it appears that even after prohibition, dietary supplements containing DMAA are still being commercialized. A rapid, simple and sensitive screening method by DART-MS was developed for detection of targeted stimulants. However, in order to avoid misidentification in casework target drugs, a confirmatory method with the optimized sensitivity toward the target compounds is required as observed in this study when comparing the two LC-MS/MS instrumentation used.

Keywords: Stimulants, DART-MS/MS and LC-MS/MS.

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Comparison of solid phase extraction and a modified QuECHERS method for quantifying fentanyl and metabolites in liver tissue

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Background/Introduction: In a typical forensic toxicological workflow, different specimens are concurrently submitted for opioid testing. These includes blood, urine, liver, kidney and other tissues. However, under special circumstances, e.g., when blood or urine are not available due to decomposition or exsanguination, liver samples are regularly the first choice for toxicological analysis mainly due to the organs ease of collection at autopsy, and homogenization for drug extraction. More importantly, the liver provides drug metabolic information that help to determine the kind of drugs that might have being used. Common practices for tissue extraction involve a solid phase extraction (SPE) or liquid-liquid extraction (LLE) but the process for these techniques can be time consuming and often involve tissue homogenizers using blenders, that could cause cross contamination. In recent years a quick, easy, cheap, effective, rugged and safe (QuECHERS) extraction protocol was introduced in the market to deal with sample with high content of fatty materials, especially for food analysis. This sample preparation technique avoids the risk of cross contamination by containing a homogenized sample into a single tube. QuECHERS has being reported as a rugged sample preparation method in blood analysis and more recently in liver tissues. In this study, we are proposing to use a simple sample homogenization system consisting of high speed mixer mill with disposable stainless steel balls contained in a home-made plastic holder. This setup helped to reduce the sample size to a few grams and improve the homogenization steps.

Objective: Compare a solid phase extraction procedure to a modified QuECHERS extraction protocol to establish a smaller sample requirement for liver tissues and reducing cost while refining the homogenization process. Additionally a confirmatory method for the target drug and metabolites was developed using liquid chromatography coupled to a triple quadrupole mass spectrometry(LC-MS/MS) method.

Method: SPE was performed using Bond Elut Certify 130 mg/3 mL columns. A modified QuECHERS extraction was used with a 0.2 g sample amount. Homogenization was achieved by the using a high-speed mixer mill (Retsch MM 200) with a home-made attachments for 1.5 mL centrifuge tubes and 4.5 mm stainless steel balls. The quantification method was performed on an Agilent 6470 Triple Quad LC-MS/MS system. Chromatographic separation was achieved on a Zorbex Eclipse Plus C18 RRHD 3.0x100 mm, 1.8 µm column with 0.1% formic acid and 5 mM ammonium formate in water (mobile phase A) and 0.1% formic acid in methanol.

Results: A validation of SPE-LC-MS/MS method for fentanyl and two known metabolites, norfentanyl and despropionyl fentanyl (4-ANPP) was performed with a limit of detection (LOD) of 0.1 ppb and an analytical measurement range (AMR) of 0.5 ppb to 250 ppb. Accuracy for all three compounds at the lowest calibrator were above 82% with a %CV of less than 5% and for the highest calibrator above 94% accuracy with a %CV less than 0.5%. R^2 values for all three compounds were all better than 0.995. The average recovery for the SPE extraction was less than 52% and the average recovery for the modified QuECHERS extraction was greater than 75%.

Conclusion/Discussion: Preserving sample volume and reducing the potential for cross contamination is a desired attribute to any forensic toxicology analysis. The procedure presented reduces the amount of sample needed to only 0.2 g. The advantage of avoiding tissue homogenizers is both time saving and reduces potential for cross contamination. Overall the QuECHERS extraction performed better than SPE while providing other benefits such as simplification of the analytical sample preparation scheme and overall improved recoveries.

Keywords: QuECHERS, Fentanyl, Liver Tissue

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Ultra-Fast Characterization of Novel Synthetic Opioids Using a Data-Independent Acquisition Analytical Workflow

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Background/Introduction: Novel synthetic opioids continue to cause widespread intoxications resulting in fatalities worldwide. As these compounds continue to surge within society, their timely and accurate detection is crucial to forensic investigators. Analysis of seized powders is highly contingent on specific and sensitive screening applications. The combination of LC separations with quadrupole time of flight mass spectrometry (QTOF-MS) gives forensic investigators a higher level of confidence in modern, synthetic opioid characterization by reliably obtaining comprehensive MS/MS spectral fragment information on every detectable component in the sample.

Objective: In this presentation, the use of data-independent Acquisition workflow for the ultra-fast and accurate identification of novel synthetic opioids present in different seized drug samples was evaluated and compared to typical GC/MS analysis.

Methods: *Sample Preparation:* Powder drug samples, seized as part of forensically relevant investigations, were aliquoted into standard autosampler vials and diluted with methanol, followed by vortex mixing to ensure homogeneity. Subsequently, these samples were diluted 1:100 in mobile phase prior to analysis. *LC-HRMS Conditions:* Analytes were chromatographically separated using a Phenomenex Kinetex C18 column (50 x 4.6 mm, 2.6 µm) column. Mobile Phase was ammonium formate in water and formic acid in methanol/acetonitrile, 500 µL/min flow rate. The QTOF-MS was operated in positive electrospray mode with independent dependent acquisition MS/MS parameters. The TOF mass scan ranged between 100-500 Da, Q1 Isolation Windows Varied between 6-34 Da and the Fragment Ion Scan Ranged between 40-510 Da.

Results : Using data-independent acquisition for analysis of seized drug samples, several novel synthetic opioids, like U-47700, furanylfentanyl, as well as other drugs of abuse, like cocaine, alprazolam, and other adulterating agents were successfully identified. U-47700 and fentanyl were accurately identified through their unique MS/MS spectra obtained within the same Q1 isolation window, even though these components share similar retention times and have closely related precursor ions. The high-quality MS/MS fragmentation generated from the data-independent acquisition workflow enabled the positive and accurate identification of heroin drug constituents like 6-MAM. This principle of identification of co-eluting species based on high resolution accurate fragment ion masses would not be feasible using less specific analytical approaches like unit resolution mass spectrometry or molecular spectroscopy.

As part of this comprehensive study, more than 500 seized drug samples, were analyzed and compared using data-independent acquisition and GC/MS acquired by full scan mode. Analytes identified by each method were compared side by side, and the unidentified compounds by a specific instrument were tallied. It was determined that more than 10% of the overall targets were missed by GC/MS analysis, but positively identified by data-independent Acquisition. When further evaluating this gap, it was found that in comparison of novel opioid identification, more than 34% of novel opioids were missed by GC/MS (e.g., furanylfentanyl).

Conclusion/Discussions: The combination of ultra-fast LC separations coupled with QTOF-MS with data-independent acquisition provides forensic investigators high-quality MS/MS fragmentation, which enables the quick and reliable identification of novel synthetic opioids (e.g., fentanyl analogues) present in seized drug samples.

Keywords: Drug Chemistry, LC-HRMS, Screening

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Evaluation of the Quantisal® II – A Novel Device for the Collection of Split Specimens of Oral Fluid

Cynthia Coulter*, Margaux Garnier, Jessica De Los Santos, James Tuyay, Jorge Daniel Sandoval, Christine Moore; Immunalysis Corporation, Pomona, CA

Introduction: Drug testing in oral fluid which requires a second, split sample be collected currently occurs by simultaneous collection (two devices in the mouth at the same time) or sequential collection. Two devices together may not collect the same volume of oral fluid, and the variability of time between collections may play a role in whether the sequential samples are of equivalent value. The evaluation of a device for simultaneous collection onto discrete separate pads is described.

Objective: To evaluate the Quantisal II for oral fluid volume collection onto two separate pads (A and B); and secondly to determine the quantitative amount of THC detected in both samples.

Methods: Firstly, to evaluate volume collected, volunteers donated oral fluid and the volume collected on pads A and B was determined gravimetrically. Secondly, THC was spiked into negative human oral fluid to determine recovery, analyte stability, and transportation stability. Finally, oral fluid samples were collected using the collection device from drug users in an approved clinical study.

Results: Quantisal II samples were collected (n=50) with a mean volume of 0.991 and 0.991 for split A and B respectively; CV 5.36% for both. The oral fluid volume on sample pads A and B was 1 mL +/-10% in all 50 donors. The difference between A and B did not exceed 15% in any pair while still meeting the range requirement of 0.900 – 1.100 mL of oral fluid. The average time for collection was 3.43 min; median = 3.0 min; range 1.00 to 9.03 min.

Drug recovery from the collection pad was determined at three different concentrations: 3, 5, and 6 ng/mL using neat oral fluid fortified with THC. Split pad collectors from Quantisal II (n=3) were dipped into solution until the blue dye indicator was activated. The pads were split and placed into separate transportation tubes containing buffer. The pads were stored overnight at room temperature then analyzed and compared to freshly prepared drug standards. Analytical results were reported for 3 replicates of each split (n=18).

Mean % recoveries for Pads A and B at 3, 5 and 6 ng/mL are as follows:

Concentration	*Pad A Mean % recovery*	*Pad B Mean % recovery*
3 ng/mL	87.09	89.71
5 ng/mL	87.63	88.40
6 ng/mL	84.42	86.77

All split pair drug concentrations were +/- 15% of each other.

THC is stable when collected with the Quantisal II device with less than 10% loss from original concentration for 5 days at 30°C and 30 days at 5°C and during routine transportation.

Specimens collected from drug users were also analyzed (n = 147). The drug concentrations ranged from 2 to 1644 ng/mL and in all cases the quantitative results from Pad A was within 15% of the THC concentration on pad B. B splits for 6 specimens were stored in their original transportation tube at 5°C and analyzed up to 3 months after original split A analysis. Samples showed less than 20% loss at 3 months and concentrations were above 40% of cutoff value (0.8 ng/mL).

Conclusion: Quantisal II device has been shown to collect 1mL +/-10% of neat oral fluid for pads A and B with a difference no greater than 15% in drug quantitation. THC is stable in the Quantisal II device in different storage conditions and during transportation. Quantisal II demonstrates equivalent collection of oral fluid onto two separate pads and represents a true "split specimen" oral fluid collection device.

Key Words: Oral fluid, Workplace drug testing, THC

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Evaluation of the Quantisal® II for Cocaine and Benzoylecgonine – A Novel Device for the Collection of Split Specimens of Oral Fluid

Cynthia Coulter*, Margaux Garnier, Jessica De Los Santos, James Tuyay, Jorge Daniel Sandoval, Christine Moore; Immunalysis Corporation, Pomona, CA

Background: Drug testing in oral fluid which requires a second split sample be collected currently occurs by simultaneous collection (two devices in the mouth at the same time) or sequential collection. Two devices together may not collect the same volume of oral fluid, and the variability of time between collections may play a role in whether or not the sequential samples are of equivalent value. The evaluation of a device for simultaneous collection onto discrete separate pads is described.

Objective: To evaluate the Quantisal II for oral fluid volume collection onto two separate pads (A and B); and secondly to determine the quantitative amount of cocaine (COC) and benzoylecgonine (BZE) detected in both samples.

Methods: Firstly, to evaluate volume collected, volunteers donated oral fluid and the volume collected on pads A and B was determined gravimetrically. Secondly, COC and BZE were spiked into negative human oral fluid to determine recovery, analyte stability, and transportation stability. All COC and BZE solutions were prepared independently because of the conversion of COC to BZE. Finally, oral fluid samples were collected using the collection device from drug users in an approved clinical study.

Results: Quantisal II samples were collected (n=50) with a mean volume of 0.991 and 0.991 for split A and B respectively; CV 5.36% for both. The oral fluid volume on sample pads A and B was 1mL +/-10% in all 50 donors. The difference between A and B did not exceed 15% in any pair while still meeting the range requirement of 0.900 – 1.100 mL of oral fluid. The average time for collection was 3.43 min; median = 3.0 min; range 1.00 to 9.03 min.

Drug recovery from the collection pad was determined at three different concentrations: 11.25, 18.75, and 22.5 ng/mL using neat oral fluid fortified with COC and BZE. Split pad collectors from Quantisal II (n=3) were dipped into solution until the blue dye indicator was activated. The pads were split and placed into separate transportation tubes containing buffer. The pads were stored overnight at room temperature then analyzed and compared to freshly prepared drug standards. Analytical results were reported for 3 replicates of each split (n=18). Mean % recoveries for Pads A and B are as follows:

Concentration	COC Pad A Mean % recovery	COC Pad B Mean % recovery	BZE Pad A Mean % recovery	BZE Pad B Mean % recovery
11.25 ng/mL	96.23	98.79	96.06	99.31
18.75 ng/mL	100.28	98.44	98.61	97.58
22.5 ng/mL	101.07	95.99	101.92	96.44

COC and BZE are stable when collected with the Quantisal II device with less than 10% loss from original concentration for 5 days at 30°C and 30 days at 5°C and during routine transportation. Specimens collected from drug users were also analyzed (n = 124). The drug concentrations ranged from 8 to 34535 ng/mL and 8 to 67171 ng/mL for BZE and COC respectively and in all cases the quantitative result from Pad A was within 15% of the concentration on pad B. B splits for two specimens were stored in their original transportation tube at 5°C and analyzed up to 3 months after original split A analysis. Samples showed less than 20% loss at 3 months and concentrations were above 40% of cutoff value (3.2 ng/mL).

Conclusion: Quantisal II device has been shown to collect 1mL +/-10% of neat oral fluid for pads A and B with a difference no greater than 15% in drug quantitation. COC and BZE are stable in the Quantisal II device in different storage conditions and during transportation. Quantisal II demonstrates equivalent collection of oral fluid onto two separate pads and represents a true "split specimen" oral fluid collection device.

Keywords: oral fluid, workplace drug testing, cocaine

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Evaluation of the Quantisal® II for Phencyclidine – A Novel Device for the Collection of Split Specimens of Oral Fluid

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Background: Drug testing in oral fluid which requires a second, split sample be collected currently occurs by simultaneous collection (two devices in the mouth at the same time) or sequential collection. Two devices together may not collect the same volume of oral fluid, and the variability of time between collections may play a role in whether or not the sequential samples are of equivalent value. The evaluation of a device for simultaneous collection onto discrete separate pads is described.

Objective: To evaluate the Quantisal II for oral fluid volume collection onto two separate pads (A and B); and secondly to determine the quantitative amount of PCP detected in both samples.

Methods: Firstly, to evaluate volume collected, volunteers donated oral fluid and the volume collected on pads A and B was determined gravimetrically. Secondly, PCP was spiked into negative human oral fluid to determine recovery, analyte stability, and transportation stability. Finally, oral fluid samples were collected using the collection device from drug users in an approved clinical study.

Results: Quantisal II samples were collected (n=50) with a mean volume of 0.991 and 0.991 for split A and B respectively; CV 5.36% for both. The oral fluid volume on sample pads A and B was 1mL +/-10% in all 50 donors. The difference between A and B did not exceed 15% in any pair while still meeting the range requirement of 0.900 – 1.100 mL of oral fluid. The average time for collection was 3.43 min; median = 3.0 min; range 1.00 to 9.03 min.

Drug recovery from the collection pad was determined at three different concentrations: 7.5, 12.5, and 15 ng/mL using neat oral fluid fortified with PCP. Split pad collectors from Quantisal II (n=3) were dipped into solution until the blue dye indicator was activated. The pads were split and placed into separate transportation tubes containing buffer. The pads were stored overnight at room temperature then analyzed and compared to freshly prepared drug standards. Analytical results were reported for 3 replicates of each split (n=18).

Mean % recoveries for Pads A and B at 7.5, 12.5 and 15 ng/mL are as follows:

Concentration	Pad A Mean % recovery	Pad B Mean % recovery
7.5 ng/mL	94.82	95.61
12.5 ng/mL	94.41	93.20
15 ng/mL	96.08	95.79

All split pair drug concentrations were +/- 15% of each other.

PCP is stable when collected with the Quantisal II device with less than 10% loss from original concentration for 10 days at 30°C and 60 days at 5°C and during routine transportation.

Specimens collected from drug users were also analyzed (n = 83). The drug concentrations ranged from 5 to 4860 ng/mL and in all cases the quantitative results from Pad A was within 15% of the PCP concentration on pad B. B splits for 3 specimens were stored in their original transportation tube at 5°C and analyzed up to 3 months after original split A analysis. Samples showed less than 20% loss at 3 months and concentrations were above 40% of cutoff value (4.0 ng/mL).

Conclusion: Quantisal II device has been shown to collect 1mL +/-10% of neat oral fluid for pads A and B with a difference no greater than 15% in drug quantitation. PCP is stable in the Quantisal II device in different storage conditions and during transportation. Quantisal II demonstrates equivalent collection of oral fluid onto two separate pads and represents a true "split specimen" oral fluid collection device.

Keywords: Oral fluid, Workplace drug testing, PCP

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Practical Considerations Using Oral Fluid Collection Devices & SPE Method Development For Drugs Of Abuse including THC And Metabolites.

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Introduction: With the resurgence of oral fluids (OF) as testing matrix for drugs of abuse (DOA) including cannabinoids, the need to provide larger and more comprehensive panels for drugs is required. However, storage buffers used in the OF collection present obstacles to low levels of detection. Isolation of DOA's from OF using SPE becomes more complicated as the choice of sorbent wash and analyte capture must be considered in order to successfully remove the emulsifying agent without disrupting analyte complexation with SPE sorbent(s).

Objective: Herein, we describe the relationship between 87 DOA's (including THC and its metabolites) and their subsequent response to the recovery and matrix effects of oral fluid buffering agents as tested with the following: water as a surrogate oral fluid, synthetic oral fluid, and patient oral fluid. Moreover, we examine the impact upon recovery and matrix effects upon modulating solvent polarity of the organic wash.

Methods: Surrogate and synthetic oral fluids were separately combined with Quantisal® buffer at a 1:3 ratio, per manufactures instructions and pretreated with 40 mL of formic acid. All oral fluid samples were extracted with 60 mg EVOLUTE® EXPRESS CX 3 mL cartridge using a Biotage® PRESSURE+ 48 positive pressure manifold. Prior to sample loading (0.5 mL) the sorbent was conditioned and equilibrated with 1.0 mL of methanol and 4% formic acid, respectively. Interferences were removed with 2.0 mL of 4% formic acid followed by 2.0 mL of various organic solvents with water at 5%, 50%, & 100% MeCN, MeOH, IPA, Acetone, DMSO, DMF, THF, and MTBE. After drying the sorbent for 5 min under 20 psi of nitrogen, analytes were eluted into 100 mL of 50 mM HCl in methanol with 2 mL of DCM/MeOH/NH_4OH [78:20:2]. Elution solvent evaporated under nitrogen at 2.0 L/min at 40°C using a Biotage® TurboVap® LV. Samples reconstituted with 100 mL of 10% methanol in 0.1% formic acid and immediately analyzed via LC/MS-MS. Analytes were chromatogrphed with an Agilent 1260 Infinity HPLC using a 50 x 3.0, 2.7 mm Restek Raptor Biphenyl column at 0.5 mL/min. Sample injection was 10 mL and nalytes eluted over a 5-minute gradient using 0.1% formic acid in methanol from 10% to 90% at 40 °C. A Sciex 4000QTRAP triple quadrupole mass spectrometer was used for direct injection/infusion of all extracted oral fluid analyses. Transitions acquired under sMRM mode under ESI positive polarity. Analytes consisted of synthetic opioids & opiates (26), benzodiazepines (13), stimulants (13), TCA's (6), anticonvulsants (4), antipsychotics (6), SSRI's (4), SNRI's (2), carbamates (2), z-drugs (2), anaesthetics (2), cannabinoid (3), NDRI (1), SARI (1), and two plant alkaloids for a total of 87 analytes.

Results: Frequency distribution analysis, using water as a surrogate OF, showed approximately 70% of analytes remained insensitive to all 12 different wash conditions. Using the Quantisal device (20 ng/mL DOA's), matrix analyses via post-column infusion (PIC) for both 50% and 100% MeOH, MeCN, acetone, and IPA wash solutions showed artifacts with +44 m/z mass spacing, strongly indicating the presence of surfactants. Under 50% wash condition the +44 m/z artifact peaks were attenuated under 50% MeCN, Acetone, and IPA and absent when using 50% MeOH. For extraction of analytes using synthetic oral fluid and Quantisal buffer, washing the CX sorbent with 50% MeOH generally produced better recoveries (73%±13) and matrix effects (12±21) with 50% IPA following at 80%±22 and 12±21%. THC, 11-OH-THC and THC-COOH recoveries increased upon use of ethylene glycol as a keeper solvent when <50% MeOH was used as a wash solvent.

Conclusions: Of the 87 analytes, both recoveries and matrix effects improved when using a 50% aqueous wash system. Although 50% aqueous washes with MeCN, IPA, or MeCN suppressed levels of surfactant breakthrough, 50% MeOH yielded the best results.

Keywords: Oral Fluids, Matrix Effects, Recovery

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Development and Validation of a New SEFRIA Immunoassay for the Detection of Opiates in Oral Fluid.

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Background/Introduction: The opiate crisis is rampant in the United States. Abuse has risen significantly over the last decades and has become the most common cause of poisoning after alcohol. Moreover, because of the uncontrolled and excessive use, prescription opiates overdoses have become a major problem and outnumbered deaths caused by traffic accidents. Opiates are alkaloids derived from the opium poppy, which interact with opioid receptors on nerve cells in the brain, such as Morphine and Codeine. They are used recreationally and medicinally where they are generally prescribed for the relief of moderate to severe pain.

Saliva is a great matrix for screening drug samples with many benefits. The collection of the sample is simple and noninvasive. Therefore, the sample collection of the subjects can be collected almost anywhere under direct supervision with a minimum risk of adulteration.

The Synthetic Enzyme Fragment Immunoassay (SEFRIA) is a homogeneous enzyme immunoassay that is an optimal platform for immunoassays that requires a low detection limit. The big advantage over traditional G6PDH–based homogeneous enzyme assay (HEIA) is the ability to have a lower cutoff with controls at ±25% of the cutoff. The SEFRIA technology is based on artificial fragments of the *E. coli* enzyme β-galactosidase, Enzyme Acceptor (EA) and Enzyme Donor (ED). The two inactive enzyme fragments reconnect to form an active enzyme (tetramer). One of the enzyme fragments (ED) is attached to the target analyte of interest and competes with the target analyte in the sample for the antibody binding site. After a short incubation to allow enzyme formation, enzyme activity is measured in the kinetic mode by the rate of change of absorbance at 570 nm, with no separation step required. The enzyme activity is directly proportional to the drug concentration in the testing specimens.

Objective: The objective of this project was to develop and validate a new highly sensitive SEFRIA immunoassay for the rapid and broad detection of opiates in human saliva.

Method: An anti-opiates polyclonal antibody-based SEFRIA immunoassay was developed and validated with LCMS confirmed saliva specimens. The assay was designed to detect opiates in oral fluid.

Results: The immunoassay is a semi-quantitative and qualitative method with a semi-quantitative reportable range of 15 to 100ng/mL and control levels at ±25% of cutoff. The cutoff of the assay will be 30ng/mL oral fluid. The qualitative precision of the assay is less than 2% CV. The SEFRIA immunoassay was validated with a total of 80 oral fluid samples previously analyzed by LCMS.

		Confirmation (30ng/mL)	
		N	P
SEFRIA™ (30ng/mL)	N	40	0
	P	0	40

The sensitivity, specificity and accuracy of the assay were found to be 100%, 100% and 100%, respectively.

Conclusion/Discussion: A highly sensitive SEFRIA immunoassay has been developed for the broad detection of opiates in human saliva. From the 80 authentic specimens the SEFRIA assay accuracy is 100% with LCMS results.

Keywords: enzyme immunoassay, opiates, Oral Fluid

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Development and Validation of a New SEFRIA Immunoassay for the Detection of Methamphetamine in Oral Fluid

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Background/Introduction: d-Methamphetamine is a powerful central nervous system stimulant that has been clinically used to treat obesity, narcolepsy and attention deficit disorder since 1930. However, due to its abuse it was subsequently placed into Control Substance Schedule II in 1971. In the 1980s, illicit methamphetamine abuse resurgence grew and now has become a major worldwide drug of abuse.

Oral fluid is a suitable alternative matrix to test drugs of abuse with many advantages over other biological matrices. Oral fluid samples are generally considered to give a better indication of current drug use, similar to blood samples, which can be especially useful for driving under the influence cases. Another advantage is the noninvasive and ease of the oral fluid collection. This advantage reduces the minimum risk of adulteration when under direct supervision.

SEFRIA is a competitive homogenous enzyme immunoassay and an optimal platform for immunoassays that require a low detection limit. This test offers an advantage over traditional G6PDH –based homogeneous enzyme assay (HEIA) due to its unique methodology, which depends on the re-association of two inactive enzyme fragments to form an active enzyme (tetramer). One of the enzyme fragments is attached to the analyte of interest and competes with the analyte in the sample for the antibody binding site. The enzyme activity is directly proportional to the drug concentration in the testing specimens.

Objective: The objective of this project was to develop and validate a new highly sensitive SEFRIA immunoassay for the rapid detection of methamphetamine in human oral fluid.

Method: An anti-methamphetamine polyclonal antibody-based SEFRIA immunoassay was developed and validated with LCMS confirmed saliva specimens. The Synthetic Enzyme Fragment Immunoassay (SEFRIA) technology is based on artificial fragments of the *E. coli* enzyme β-galactosidase, Enzyme Acceptor (EA) and Enzyme Donor (ED). The EA Reagent, containing EA protein and antibody, is mixed with sample, and any drug present in the sample binds to the antibody. The ED reagent, containing ED-drug conjugate and CPRG substrate, is then added, and the conjugate either binds to the remaining antibody, or reacts with EA to form active enzyme in proportion to the drug present in the sample. After a short incubation to allow enzyme formation, enzyme activity is measured in the kinetic mode by the rate of change of absorbance at 570 nm, with no separation step required. The assay was designed to detect methamphetamine in oral fluid.

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Results: The immunoassay is a semi-quantitative and qualitative method with a semi-quantitative reportable range of 25 to 200ng/mL and control levels at ±25% of cutoff. The cutoff of the assay was determined to be 50 ng/mL. The qualitative precision of the assay is less than 2% CV. The SEFRIA immunoassay was validated with a total of 82 oral fluid samples previously analyzed by LCMS.

		Confirmation (50ng/mL)	
		N	P
SEFRIA™ (50ng/mL)	N	38	0
	P	0	44

The sensitivity, specificity and accuracy of the assay were found to be 100%, 100% and 100%, respectively.

Conclusion/Discussion: A highly sensitive SEFRIA immunoassay has been developed for the broad detection of methamphetamine in human saliva. When applied to authentic specimens the assay correlated well with LCMS results.

Keywords: enzyme immunoassay, methamphetamine, Oral Fluid

P94

Development of A New rFab-Based Homogeneous Immunoassay for the Highly Specific Detection of Tapentadol in Urine

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Background/Introduction: The majority of deaths (over 50,000 in 2016) that are caused by drug overdose are related to the potential abuse of prescription pain medications. A significant effort has been made to develop highly specific immunoassays (IAs) as quick and inexpensive methods for determining the presence of prescription drugs such as oxycodone, fentanyl and tramadol in urine to meet compliance requirements. Tapentadol was a new and synthetic analgesic approved in 2008 (immediate-release) and 2011 (extended-release) for acute and chronic pain management. In 2014, the first polyclonal-based tapentadol homogeneous immunoassay was developed. Certain cross reactivities of the selected polyclonal antibody to amphetamines and tramadol was observed because of their structural similarity, which sometimes yielded false positive results. In order to remove these undesirable cross reactivities, a new recombinant fragment antigen binding (rFab) antibody was developed, which resulted in a significant improvement on assay performance by almost completely removing undesired cross reactivities (Table 1).

Objective: Recently, rFab antibodies have been used in many US FDA approved drugs (>70B annual sales) for treating a variety of cancers, but are very limited in IVD products, especially small molecule immunoassays, mainly due to the high complexity and cost of rFab development. There have been several successfully developed, novel rFab-based immunoassays for oxycodone, PCP, EDDP and 6AM with unprecedented specificity when compared with conventional monoclonal antibodies-based immunoassays. After extensive positive/negative selection processes, a highly specific rFab antibody for tapentadol has been obtained and applied to homogeneous immunoassay (HEIA) for the detection of tapentadol and its major metabolites in urine.

Method: An anti-tapentadol recombinant Fab antibody-based HEIA immunoassay was developed and validated with LCMS confirmed urine specimens. The assay was designed to detect tapentadol in urine.

Results: The HEIA is based on the competition of tapentadol-labeled enzyme glucose-6-phosphate dehydrogenase (G6PDH) conjugate with the free drug in the urine sample for the fixed amount of tapentadol antibody binding sites that inhibit enzyme activity after binding enzyme conjugate. This creates a dose-response relationship between the drug concentration in the urine and enzyme activity. With a cutoff at 200ng/mL, the false positive rate dropped from 39% for the polyclonal antibody-based assay to 2% for the rFab-based assay when 95 tramadol positive urine specimens were used to challenge the assay specificity.

Table 2. Comparison between pAb and rFab with 95 Tramadol Positive Urine Specimens

		pAb (200ng/mL)		rFab (200ng/mL)	
		+	-	+	-
LCMS (200ng/mL)	+	0	0	0	0
	-	37	58	2	93

Table 1. Removing Cross Reactivities by rFab

Conc. (100K ng/mL)	Polyclonal (ng/mL)	rFab (ng/mL)
Cyclobenzaprine	171	1
Diphenhydramine	188	20
d-Methamphetamine	114	-3
PCP	102	-5
Tramadol	177	4
N-Desmethyl tramadol	109	1

Conclusion/Discussion: A highly sensitive and specific HEIA based on a new rFab antibody for tapentadol in urine has been developed. The accuracy has been validated with 209 urine samples and found to be 99%. These results demonstrate that an rFab antibody could be used in a small molecule immunoassay with far superior performance than polyclonal antibody-based immunoassays, which will help greatly with cost and efficacy of urine pain management program.

Keywords: enzyme immunoassay, tapentadol, urine

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Development of a Rapid (Less Than Twenty Three Minutes) Biochip Based Multi-Analytical Detection of Drugs Of Abuse In Whole Blood

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Background/Introduction: In blood drug testing is beneficial to increase the information provided in the screening step from a single sample as it facilitates the testing process. Biochip array technology enables the multi-analytical screening of drugs, which increases the screening capacity. The application of the biochip based immunoassays to the Evidence MultiSTAT analyser allows rapid drug screening which is relevant in test settings.

Objective: This study aimed to develop a rapid (less than 23 minutes) and simple multi-analytical biochip based screening of twenty drug classes, including new psychoactive substances, from a single sample of blood. The initial analytical evaluation is presented.

Method: Based on biochip array technology, simultaneous biochip based immunoassays were applied to the fully automated biochip analyser Evidence MultiSTAT. This system processes a self-contained cartridge containing all the components required for the immunoassay reactions**.** After simple centrifugation and dilution (1:4) the blood sample is ready to be added to the biochip. The blood sample is tested against a cut-off sample, the results are qualitative.

Repeatability was determined by preparing and assessing sample replicates at +50% of the cut-off and -50% of the cut-off against a cut-off sample. The samples were analyzed twice per day for 10 days. The qualitative results were determined and presented as percentage agreement. Accuracy was determined as follows: 100 urine samples were used: 50 negative samples and 50 spiked samples (spanning the cut-off). Each sample was run against a cut-off sample, a qualitative result was determined for each of the twenty drug classes and the results presented as percentage agreement.

Results: Simultaneous immunoassays and cut-offs applied: 5 ng/mL AB-CHMINACA, 2 ng/mL AB-PINACA, 5 ng/mL alpha-pyrrolidinopentiophenone (alpha-PVP), 50 ng/mL amphetamine, 50 ng/mL barbiturates (standardized to phenobarbital), 20 ng/mL benzodiazepines (standardized to oxazepam), 25 ng/mL benzoylecgonine/cocaine, 2 ng/mL buprenorphine, 500 ng/mL ethyl glucuronide (ETG), 1 ng/mL fentanyl, 10 ng/mL 6-monoacetylmorphine (6-MAM), 10 ng/mL methadone, 50 ng/mL methamphetamine, 80 ng/mL opiate (standardized to 6-MAM), 10 ng/mL oxycodone, 1,000 ng/mL pregabalin, 10 ng/mL propoxyphene, 5 ng/mL tramadol, 60 ng/mL tricyclic antidepressants (TCAs, standardized to nortriptyline), 10 ng/mL tetrahydrocannabinol (THC). Initial analytical evaluation of repeatability and accuracy showed a percentage agreement >90% and >80% respectively for all the assays.

Conclusion/Discussion:

Data indicate that twenty drug classes can be screened in less than 23 minutes from a single blood sample by applying simultaneous biochip-based immunoassays to the biochip analyser Evidence MultiSTAT. This application will facilitate the drug testing process as it allows a rapid and easy to use multi-analytical screening of blood samples.

Keywords: Biochip array, drugs testing, rapid blood screening

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Evaluation of Matrix Component Removal using Novel Flow-Through Scavenging Plates or Columns for Drugs of Abuse Testing in Urine.

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Background/Introduction: Dilute and shoot (D/S) is the most common form of sample preparation for the analysis of drugs of abuse in urine. High analyte cutoffs combined with sensitive mass spectrometers allow substantial sample dilution while still reaching desired limits of quantitation. However, this technique presents various issues resulting in increased MS downtime. This poster evaluates the extraction of a range of drugs of abuse from hydrolysed and non-hydrolysed urine using a novel flow-through matrix scavenging plate or column. Specific investigation of matrix component removal in terms of creatinine and urea, salt residue, pigmentation associated with urobillin content and protein removal will be demonstrated.

Objective: The objective was to maximise the removal of components in non-hydrolysed urine and enzymatic-hydrolysed urine, while still recovering common drugs of abuse for LC-MS/MS analysis.

Method: LC-MS/MS analysis was performed using a Waters ACQUITY UPLC system coupled to a Quattro Premier XE triple quadrupole mass spectrometer. Optimized MRM transitions were selected using the most intense precursor/product ions. A panel of drugs of abuse were chromatographed using a Restek Raptor Biphenyl HPLC column, while alternative chromatography for creatinine and urea removal was provided using a Betamax acids column. Protein removal from hydrolysed urine was investigated via gel electrophoresis using a NuPAGE Novex 12% bis-tris mini gel with MOPS SDS running buffer. Gels were run for approximately 65 minutes and compared to a protein benchmark molecular weight ladder.

Results: Data compared traditional dilute-and-shoot approaches to the performance of the flow-through matrix-scavenging plate or column. D/S was performed using 100 µL of hydrolysed or non-hydrolysed human urine, diluted to 1 mL with aqueous buffers. Scavenging performance was evaluated using 100-250 µL of hydrolysed or non-hydrolysed urine mixed with 600 µL of ACN followed by filtering through the plate. Performance was initially evaluated visually for salt and pigment removal. Post evaporation demonstrated effective removal of both matrix components. Creatinine and urea investigation typically demonstrated removal of greater than 90% for both analytes. Gel electrophoresis testing compared β-glucuronidase, raw and hydrolysed urine before and after dilution and hydrolysed urine processed using the plate. The enzyme adds substantial protein content to the matrix typically between 60-220 kDa. Although dilution lessens this content subtle bands were still visible. The scavenging plate completely removed all protein when using 100-250 µL of hydrolysed urine. Full scan and post column infusion experiments showed substantial matrix clean up compared to D/S whether direct injection of the extract or evaporation and concentration of the scavenging column or plate was employed. Analyte recoveries were observed between 50-90% with corresponding RSDs below 10%. Lower matrix suppression resulted in signals capable of reaching cut-off levels of all drugs of abuse classes tested. Calibration curves were constructed using spiked urine from 1 to 400 ng/mL. Results demonstrated good linearity and coefficients of determination (r^2) values greater than 0.99 for all analytes. Following direct injection (1 µL) of the eluate most analytes demonstrated LOQs at low or sub ng/mL levels. Greater sensitivity was achieved through extract evaporation and concentration.

Conclusion/Discussion: This poster demonstrates the use of a novel approach for the extraction and cleanup of a range of DoA from hydrolysed and non-hydrolysed urine. The technique provides excellent removal of many of the interfering components associated with urine drugs of abuse testing resulting in less frequent instrument cleaning, column replacement and associated downtime compared to dilute and shoot approaches

Keywords: Urine, Drugs of abuse, Matrix-scavenging

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Analysis of Synthetic Fentanyl Analogs in Whole Blood Using Mixed-Mode SPE and UPLC-MS/MS for Forensic Toxicology

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Background/Introduction: Overdose deaths from opiates and synthetic opioids have risen substantially over the past few years, with the largest increases attributed to synthetic opioids such as fentanyl and its analogs. [1] Due to their high potency, the concentrations of these compounds are often in the sub ng/mL range. In addition, forensic samples are often analyzed from whole blood, presenting challenges not seen in plasma or urine samples. This work details a rapid UPLC-MS/MS method for the analysis of synthetic fentanyl compounds in whole blood employing a novel mixed-mode SPE strategy designed to minimize residual phospholipids. The resulting method minimizes ion suppression while producing linear and accurate quantification over the entire expected analytical range of these compounds.

Objective: The objective of this study was to develop a rapid method for quantitative confirmation of synthetic fentanyl compounds in whole blood that minimizes residual phospholipids.

Methods: One hundred microliters of whole blood calibrators, QC or case samples were diluted 1:1 with 100 mM $ZnSO_4$/NH_4OAc followed by precipitation with 1:1 ACN:MeOH. After centrifugation, the supernatant was diluted with 1 mL 4% H_3PO_4 and loaded directly onto Waters Oasis PRiME MCX µElution plates. The sorbent was washed with 200 µL 2% formic acid:100 mM NH_4COOH followed by 200 µL MeOH. Samples were eluted with 2 x 25 µL aliquots of 50:50 ACN:MeOH containing 5% strong ammonia solution (28%) and diluted with 50 µL of 97:2:1 water:ACN:formic acid. Five microliters was injected on to a Waters Xevo TQ-S micro tandem quadrupole MS system. Analytes were chromatographically separated on a 1.7 µm Waters BEH C_{18} column (2.1 x 100 mm). The method was evaluated for recovery, matrix effects, linearity, accuracy, precision, interferences, sensitivity, carryover, and dilution integrity.

Results: Sixteen synthetic fentanyl analogs including carfentanyl, furanyl fentanyl and acetyl fentanyl were extracted and analyzed from whole blood with recoveries that ranged from 63-87% with an average of 82%. All %CVs for recovery were <10%. Mean matrix effects were all less than 20%. The SPE protocol was able to eliminate >95% of residual phospholipids compared to traditional mixed-mode cation exchange SPE. This was determined by scanning for precursors of *m/z* 184.2, a choline group common in fragmented phospholipids, and comparing the total area of these between the two sample preparation procedures. The UPLC-MS/MS method was rapid, with a 3 minute analysis and a total cycle time of less than 4 minutes. All analytes demonstrated linearity from 0.050 ng/mL to 20 ng/mL, easily encompassing previously documented levels in whole blood samples.[2] Quality control samples at 0.075, 0.75, 7.5 and 15 ng/mL were accurate and precise. Individual accuracy values ranged from 91-112% and between run averages (*N*=5) ranged from 94-105%. All individual %CV values were <9%. Authentic post-mortem case samples (with 3 additional synthetic fentanyl analogs) were analyzed using this method and showed good correlation with an established method from a reference laboratory.

Conclusion: The combination of novel SPE cleanup and UPLC-MS/MS analysis resulted in a method capable of rapidly and accurately analyzing a wide variety of synthetic fentanyl analogs from whole blood. Residual phospholipids were minimized without compromising assay performance compared to traditional mixed-mode SPE procedures. The use of µElution plates enabled direct analysis without any evaporation or reconstitution resulting in a simple, rapid and robust procedure.

Keywords: Synthetic fentanyl analogs, Solid Phase Extraction (SPE), blood

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POSTER

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False-Positive Cocaine Immunoassay Screening Results from Hair Suspected to Have Been Treated with a Pink Semi-Permanent Hair Coloring Product

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Background/Introduction: Hair testing for drugs of abuse (DOA) is currently the only available method that provides detection of up to a 90-day repetitive drug usage. Hair care product application and cosmetic treatments, including hair coloring, can influence the stability of drugs incorporated into hair, as well as hair structure, and can lead to presumptive false-negative and/or false-positive screening results. A single hair specimen screened positive for cocaine during performance characterization studies of the HairCheck-DT (Cocaine) ELISA,that upon confirmation by GC-MS was negative for cocaine. Repeat testing confirmed its false-positive status. The specimen extract was bright pink in color when pipetted onto the ELISA plate and also yielded false-positive results when used on other HairCheck-DT ELISA kits.

Objective: To determine if certain hair care products or cosmetic treatments (especially semi-permanent hair coloring and cationic dyes) can lead to presumptive false-negative and/or false-positive screening results in ELISA for hair drug testing.

Method: De-identified cocaine-positive and-negative hair specimens were weighed for ELISA and GC-MS analysis and prepared for testing by standard practices. Screening for the presence of cocaine in the hair specimens was performed using the HairCheck-DT (Cocaine) ELISA kit, with a cut-off concentration for cocaine and its metabolites at 300 pg/mg. GC-MS confirmation of cocaine and metabolites was conducted using solid-phase extraction and derivatization. Replication of the false-positive "pink hair" phenomenon was accomplished using commercially available semi-permanent hair-coloring creams on negative donor hair. Constituent cationic dye components contained in the hair-coloring creams were then used in interference studies on HairCheck-DT (Cocaine) ELISA to elucidate which specific dye components could cause a presumptive false-positive response on the screening assay. Serial dilutions of suspect candidate dyes were compared to the extracted false-positive "pink hair" specimen to identify concentration that caused false positive interference by spectrophotometric scanning.

Results: Certain cationic dyes found in semi-permanent hair coloring products can potentially generate presumptive false-positive results in a cocaine screening ELISA. Identification of the mechanism of action for cationic dye interference in the ELISA was beyond the scope of this study. However, direct and specific involvement of the cocaine antibody, used in coating the microplates for the competitive ELISA, can be ruled out, since the offending hair specimen also produced false-positive results with other HairCheck-DT ELISA kits. In the case of the "pink hair specimen" a signature spectrum was observed in the unknown donor sample that corresponded to a cationic dye component known as Basic Violet 16. No other peak was observed in this spectrophotometric analysis.

Conclusion/Discussion: To our knowledge this study represents the first report that certain semi-permanent hair-coloring products can lead directly to a presumptive false-positive immunoassay screening result through interference with fundamental aspects of the ELISA. Constituent dyes in the hair care products lead to negative samples appearing positive for the tested drug. However, confirmatory testing by GC-MS reveals the true drug status of the hair samples. Our own incidence studies indicate 2% of the submitted hair sample population show evidence of being subjected to semi-permanent hair coloring, moreover, any such screening false-positives generated would be sent to confirmation testing and confirmed negative. This study establishes that the incidence of semi-permanent hair coloring interfering with DOA tests is relatively low, and confirmatory testing by GC-MS (or similar technology) establishes the true drug status of the hair samples.

Keywords: enzyme-linked immunosorbent assay; cosmetic treatment; hair analysis