SOFTEMBER ABSTRACTS

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A 4-Year Systematic Analysis of Commercial E-liquids: The Evolution of G.R.A.S. to Inhaled Toxins

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Background/Introduction:

Electronic cigarettes (e-cigs) were originally developed as an alternate method for nicotine delivery. The e-liquids are made of ratios of humectant formulations, pharmacologically active ingredients, flavoring chemicals, volatiles, preservative chemicals, and other "enhancing" chemicals. E-cigs have also been adopted for drugs other than nicotine (DOTN), including cannabidiol (CBD) and tetrahydrocannabinol (THC). E-liquid manufacturers use chemicals that are generally regarded as safe (GRAS) for oral consumption. The FDA promulgated regulations to govern e-cigarette devices and e-liquids in May 2016, yet product approval deadlines are May 2020. The flavoring bans that were instituted in January 2020 only govern pod-based products. E-liquid formulations for re-filling e-cigarettes have not been heavily scrutinized by regulatory agencies, under the auspice that only pod-based products are most likely to be used by children. E-liquid formulations have evolved as a result of vaping preferences, public health sentiment, and looming regulations. Quality assurance and product safety remain inadequate, demonstrated recently by e-cigarette or vaping product associated lung injury (EVALI).

Objectives:

The objective of this study was to evaluate the chemical composition of e-liquids and to establish over time humectant formulations, pharmacologically active ingredients, flavoring chemicals, volatiles, preservative chemicals, and other "enhancements".

Methods:

Construction design of the pods/cells were documented prior to e-liquid analysis. Undiluted e-liquids were screened using AccuTOF Direct Analysis in Real Time Mass Spectrometry (DART-MS). Glycol concentrations and general unknown screening were performed using gas chromatography-mass spectrometry (GC-MS). Volatiles were evaluated by headspace gas chromatography with a flame ionization detector (HS GC-FID).

Results:

E-liquids were found to have varying ratios of PG and VG, ranging anywhere from 100% PG to 100% VG. Newer e-liquids contained polyethylene glycol (PEG), diacylglycerols, and various mid-chain triglycerides (MCTs). Pharmacologically active ingredients included nicotine, minor tobacco alkaloids, caffeine, mitragynine, paynantheine, cannabinoids, synthetic cannabinoids, gamma-butyrolactone (GBL), and dextromethorphan. Multiple flavorants included terpenes, pyranones, aldehydes, and some esters. Preservatives included butylated hydroxytoluene, benzoic acid, decanoic acid, and triethyl citrate. Volatiles included: methanol, ethanol, acetone, and isopropanol. Chemical enhancements included Vitamin E, gamma-tocopherol, phytol, olivetol, and flavorant-PG adducts.

Conclusion/Discussion:

Surveillance shows that PG and VG are still commonly used, but an expansion of chemicals used as humectants was observed. Alternative pharmacologically active ingredients also expanded, while the legal cannabis industry helped drive e-cigarette design. Over time, vanillin and ethyl maltol (vanilla and caramel) are the most prevalent identified flavorants, followed by mint and fruit flavorants. Volatiles continue to be a component of e-liquid formulations. Ethanol is common as a solvent for flavorants or psychoactive compounds. Chemicals that are food-grade preservatives, vitamins, organic acids, and terpenoids have become more prevalent in recent years. In the vacuum of strong and clear federal regulations, the e-cigarette industry has rapidly expanded product types. Formulations are made of chemicals that are considered GRAS by the FDA, which has generally provided an aura of safety for consumers. However, the recent EVALI epidemic exposed the toxic nature of vaping. The e-cig industry has facilitated the public consumption of recreational drugs and the abuse of these substances by providing efficient drug delivery devices.

A Decade of Fentanyl Related Overdoses in San Francisco

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Background/Introduction:

Fentanyl is now most widely known for its national trends as an illicit recreational drug among individuals with opioid use disorder. In 2017, fentanyl was classified as the number one drug involved in drug overdoses in the United States, killing an average of 8.7 out of every 100,000 people. Problematic more so is the addition of fentanyl into, or consumed with, other drug products can exacerbate CNS depression, particularly to opioid naive individuals who may be unaware of such additions.

Objectives:

It was the goal of this research to offer insight to accidental overdose trends involving sole fentanyl consumption, as well as co-consumption with medicinal opioids, heroin, methamphetamine, and cocaine in San Francisco from 2009-2019.

Methods:

Biological samples were analyzed through various methods over the studied period. Screening methods included ELISA and Biochip. Confirmation and quantitative methods included GC-MS and LC-MS/MS. All investigative, toxicological and autopsy data for this study was sourced from the final reports. In addition, the following demographic information was collected: race/ethnicity, age, sex, time of death, date of death, and zip code where the decedent was pronounced deceased. Inclusion criteria for this study included only accidental overdose cases that contained at least one of the following compounds of interest: fentanyl, norfentanyl, 6-monoacetylmorphine, morphine, codeine, hydrocodone, hydromorphone, oxycodone, oxymorphone, methadone, tramadol, buprenorphine and norbuprenorphine. All studied cases where the decedent was admitted to the hospital and received medicinal fentanyl were also excluded for the purpose of this study.

Results:

From 2009-2019, over 3,400 total cases were positive for either fentanyl, heroin, medicinal opioids, cocaine, methamphetamine, or any combination thereof. Approximately 60% of those cases were determined as accidental overdoses. More than half of accidental overdose cases directly attributed poly-drug consumption to the cause of death rather than isolated use. From 2009-2016, there was an increase of 163%, followed by a sharp increase of 1010% from 2016-2019, resulting in an overall increase of 2813% from 2009-2019. In 2019, accidental overdose deaths were predominately due to the use of fentanyl, followed by methamphetamine and cocaine. Males made up over 70% of all accidental overdoses, and those of white and black ethnicity were overrepresented (91%) compared to the ethnic make-up of the community. The 55-64 year-old population was most affected overall however, fentanyl-related deaths were more prominent in the 25-34 year-old age group.

Conclusion/Discussion:

This comprehensive study shows that fentanyl-related deaths have been increasing, demonstrating continued workload demands that forensic toxicology laboratories are facing. It is expected that surrounding cities, counties and states on the West Coast should also be experiencing similar trends. Early implementation of extensive naloxone programs within the community have significantly reduced the amount of overdose fatalities, with thousands of survived overdoses being recorded each year due to such efforts.

A Rapid and Reliable LC-MS-MS Method for Cannabidiol (CBD) and 11-nor-9-Carboxy-THC in Urine

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Background/Introduction:

Recently, there has been an increase in the legality of cannabis in the United States and internationally. Legalization and usage of hemp is also expanding and increasing. The Agriculture Improvement Act of 2018 removed hemp-derived products from the list of Schedule I substances on the controlled substances list. This policy change has seen an increase in popularity of cannabidiol (CBD) products being proposed to have therapeutic benefits. CBD is the significant component of many cannabis and hemp products. Our laboratory is a high production drug testing laboratory serving physicians managing patients with chronic pain and patients in drug treatment facilities. These physicians have requested CBD testing of their patients either due to patients taking CBD products on their own, or the physician suggesting to their patients to take CBD products.

Objectives:

A rapid, reliable, and sensitive method for cannabidiol (CBD) and 11-nor-9-Carboxy-THC (THC-COOH) was developed and validated to analyze our client's request for CBD testing in urine samples of their patients.

Methods:

Working calibrators and positive control containing CBD and THC-COOH, and a hydrolysis control containing THC-COOH glucuronide were prepared in drug free urine from purchased standards. Stock Internal Standard (IS) was prepared in methanol with CBD-D3 and THC-COOH-D9. Samples were prepared with a Tecan Evo 100 automated aliquotor in a 96 well plate using 300 µL of sample and 110 µL internal standard hydrolysis solution (44.6% IMCS Inc. β-glucuronidase enzyme solution, 48.9% IMCS buffer, 6.52% IS stock solution). After a 30-minute room temperature hydrolysis step, extraction was performed using an Integra VIAFLO 96 with DPX Technologies solid phase extraction (SPE) tip. Samples were eluted and injected onto an Agilent 6490 LC-MS-MS with Agilent 1260 Infinity HPLC using electro-spray ionization. Total run time was 3.2 minutes, with starting conditions of 20:80 mobile phase A (5 mM ammonium formate in 0.01% formic acid in water): mobile phase B (0.01% formic acid in methanol) and separation was achieved using a gradient to 100% mobile phase B at 1.5 minutes. Injection volume was 7 µL. Dynamic multiple reaction monitoring (dMRM) acquisition method was used for all transitions. Two transitions were monitored for the analyte and one transition for the internal standard.

Results:

The assay was linear from 0.5-80 ng/mL for CBD and 5.0-800 ng/mL for THC-COOH (five replicates of seven concentrations). The Carryover limit was the Upper Limit of Linearity (ULOL). The cutoff was set at 1 ng/mL for CBD and 10 ng/mL for THC-COOH. Precision was less than 15% (ten replicates per day, three days, four concentrations). Accuracy (same data as precision) showed the mean of the replicates to be within ± 20% of the expected concentration. No interference was demonstrated with Δ-9-THC, Δ-8-THC, 11-hydroxy-THC, cannabinol, cannabigerol and an over-the-counter mixture. The matrix effect for CBD was -75% and for THC-COOH was 27% (pooled fresh urine from ten volunteers versus mobile phase). Sample stability was shown to be 10 days for CBD and 14 days for THC-COOH at room temperature. Nine patient samples were analyzed. Two patients were previously analyzed at a reference laboratory (CBD positive/THC-COOH negative); our method found the same results. Two samples were from known CBD users; our method found each of these samples positive for CBD and negative for THC-COOH. Five samples were previously analyzed for THC-COOH (all positive) by our laboratory's routine confirmation method; our CBD/THC-COOH method found each of these samples positive for THC-COOH and negative for CBD.

Analysis of Kratoms Main Psychoactive Components: Mitragynine and 7-Hydroxymitragynine

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Background/Introduction:

Kratom (Mitragyna Speciosa) is a plant native to Southeast Asia that is typically consumed as a pill, capsule or extract and is reported to induce stimulant and opioid-like analgesic effects in the user in a dose- and time-dependent manner. Although highly popular as an unscheduled recreational drug, its high potential for abuse have raised considerable safety and health concerns. As a result, effective monitoring of these active alkaloids in urine specimens can provide health professionals a clearer picture of consumption trends of this drug and its evolution in the recreational consumer market.

Objectives:

The objective of this study is to develop a quantitative detection method for the analysis of kratom's main components, mitragynine and 7-HMG in urine samples using the QTRAP 4500 system. The combination of a simple dilute-and-shoot protocol and a targeted confirmation method using multiple reaction monitoring (MRM) MS/MS detection provided excellent sensitivity and linearity for the identification of these analytes in human urine specimens.

Methods:

A stock solution of mitragynine and 7-HMG was prepared at various concentrations in methanol. The resulting solutions were subsequently diluted in control human urine to create calibrator solutions and mixed with a hydrolysis buffer and a recombinant β-Glucuronidase solution for hydrolysis. The hydrolyzed mixtures were then centrifuged and the supernatant was transferred to an autosampler vial for analysis by LC-MS/MS. Mitragynine and 7-HMG were chromatographically separated using a Phenomenex Biphenyl (2.6µm, 3 x 50mm) column. Mass spectrometric detection was conducted on a QTRAP 4500 System operating in positive electrospray mode using a scheduled multiple reaction monitoring (MRM) algorithm to ensure acquisition of an adequate amount of data points for quantifiable data.

Results:

Control human urine samples were spiked with mitragynine and 7-HMG at concentrations ranging from 2 to 500 ng/mL. These standard urine mixtures were spiked with the internal standard mixture, hydrolyzed using the aforementioned procedures, subjected to a final dilution step and injected to build a data processing method. Each calibration levels was evaluated over three replicates. The lowest level of quantitation (LLOQ) of 2 ng/mL showed a quantifiable peak with a signal-to-noise ratio (S/N) greater than 10. These signals suggest that the limits of detection (LOD) have the potential to be lower. Calibration curves for both analytes produced good linearity with correlation (R2) of greater than 0.99 across the 2.5 orders of concentration studied. Precision and accuracy were established using five spiked matrix samples at two levels (10 and 200 ng/mL). Acceptance criteria of ≤ 20% CV (coefficient of variation) for precision and accuracy of ± 20% was used for the analysis. All samples yielded acceptable results. Carryover was evaluated by injection of a blank following a sample containing 500 ng/mL (ULOQ) of each analyte. No carryover was observed for either analyte in the method at that concentration. It was determined that samples with concentration above the ULOQ should be diluted and re-analyzed to ensure measurement within the linear dynamic range of the assay.

Conclusion/Discussion:

In this study, a quantitative MRM method for LC-MS/MS analysis of kratom components, mitragynine and 7-HMG, was successfully developed using the QTRAP 4500 System. A simple dilute-and-shoot protocol was easily implemented to extract the analytes from human urine samples. The developed method provided excellent sensitivity and linearity for the identification of these analytes in a human urine specimen. In addition, the assay showed good sensitivity down to the 2 ng/mL level in urine samples and acceptable precision and accuracy were achieved at each concentration evaluated when five spiked urine samples were evaluated. Overall, the described workflow provided excellent sensitivity and linearity for the identification of mitragynine and 7-HMG in human urine specimen.

Automated Hydrolysis, Extraction and Analysis of Synthetic Cathinones in Urine using a Robotic Autosampler and LC/MS/MS Platform

Authors and Affiliations: Fred Foster* and Jack Stuff GERSTEL, Inc., Linthicum, MD

Background/Introduction:

Synthetic cathinones (also known as Bath Salts) are a group of drug compounds designated as “new psychoactive substances” (NPS). They are unregulated, mind-altering substances with no approved medical use. Since they are cheap substitutes for other stimulants like methamphetamine and cocaine, users will unfortunately turn to these addictive and dangerous alternatives to achieve their euphoric effects. There is a critical need for forensic, heath care, and law enforcement scientists to be able to quickly assess and monitor which synthetic cathinone is involved, in order to effectively respond to cases involving these compounds.

Objectives:

In this report, the complete automation of an enzymatic hydrolysis procedure coupled with the online extraction and LC/MS/MS analyses of the synthetic cathinone compounds mephedrone and MDPV in urine is discussed.

Methods:

Using the GERSTEL MPS robotic autosampler, syringe transfer of all liquids involved in the enzymatic hydrolysis procedure, controlled mixing of the samples for a defined period of time, as well as extractions of the subsequent hydrolyzed urine samples using dispersive solid phase extraction were performed. The resulting eluents from the automated extractions were then introduced into the Agilent Ultivo LC/MS/MS instrument.

Results:

As a result of this study, we were able to show that an automated enzymatic hydrolysis and subsequent cleanup method was successful using the GERSTEL MPS robotic sampler for synthetic cathinones from a 100 uL urine sample. Linear calibration curves from 2.5 to 1000 ng/mL resulting in R2 values 0.99 or greater were achieved upon the complete automated hydrolysis procedure. The results from this automated method proved to be accurate and precise. Accuracy data averaged 99.4% (range: 95.7% -102%) and precision data averaged 6.04%CV (range: 2.59% -19.3%) for all synthetic cathinone compounds analyzed. Coupling the dispersive solid phase extraction to the LC/MS/MS minimizes matrix interference from these biological samples.

Conclusion/Discussion:

Using this method, synthetic cathinone analytes can be rapidly and reproducibly isolated from hydrolyzed urine samples using an automated cleanup procedure coupled to LC/MS/MS analysis using the Agilent Ultivo Triple Quadrupole Mass Spectrometer, allowing their respective limits of detection to be met. Automating the entire hydrolysis, extraction, and subsequent analysis by LC/MS/MS provides the critical high throughput analysis for synthetic cathinones in urine.

Benzodiazepines and Z-drugs prevalence from 2015 – 2019 on Sexual Assault, DUID, and Postmortem Cases

Authors and Affiliations: Helen H. Ha Orange County Crime Lab, Santa Ana, CA

Background/Introduction:

Benzodiazepines and other sedative drugs are commonly prescribed drugs to treat insomnia and anxiety. Due to their multiple functionalities, they are often abused. Poly-drug abusers and opioid dependents also use them to ease the "crash" after ingestion. Tracking these drugs prevalence is crucial for policy makers as they decide on analysis requirements for drug-facilitated sexual assaults, driving under the influence of drugs (DUID), or death investigations.

Objectives:

The objective is to provide an overview of drug prevalence for benzodiazepines and sedative hypnotics over a period of five years. The quantitative method includes alprazolam, chlordiazepoxide, clobazam, clonazepam, diazepam, diphenhydramine, doxylamine, estazolam, etizolam, flunitrazepam, flurazepam, hydroxyzine, lorazepam, midazolam, nordiazepam, oxazepam, phenazepam, temazepam, triazolam, zaleplon, zolpidem, and zopiclone. The qualitative method includes 2-hydroxyethylflurazepam, 7-aminoclonazepam, 7-aminoflunitrazepam, alpha-hydroxyalprazolam, alpha-hydroxytriazolam, bromazepam, chlordiazepoxide, demoxepam, desalkylflurazepam, diazepam, nitrazepam, nordiazepam, oxazepam, temazepam, and suvorexant.

Methods:

Method was previously validated and published following SWGTOX guidelines for limit of detection, limit of quantitation, bias and precision, stability, interference studies, ionization suppression/enhancement and carryover. All blood samples were collected from Orange County Crime Lab, Santa Ana, California. Sample, blank, and standards required 250 µL into a culture tube. Urine samples were hydrolyzed using IMCSzyne β-glucuronidase mixed with IMCS® pH 7.4 rapid hydrolysis buffer at 55 °C for 30 minutes. Next, 50 µL of internal standard and 750 µL of acetonitrile were added prior to vortexing and centrifugation for 5 minutes at 2500 rpm. The supernatant was transferred to test tubes, aspirated on DPX®WAX tips by a pneumatic extractor. 50 µL of aspirant transferred to LC vials with 800 µL of initial mobile phase. A Waters Xevo TQ-S with Acquity UPLC with Waters BEH C18 1.7 µm, 2.1 x 100 mm column, held at 40 oC, was used. Organic mobile phase was acetonitrile with 0.1% formic acid and aqueous mobile phase was ultra pure water with 0.1% formic acid. Two MRM transitions were collected for each drug for identification and quantitation when compared to a calibration curve.

Results:

Out of 47,116 cases received from 2015 to 2019, 6969 were scheduled for confirmation of benzodiazepines and sedative drugs; 5710 cases were positive for the drugs present in the method. DUID cases made up of 62.1%, 28.5% for death investigation, 2.2% for sexual assaults and 7.2% with other charges. Over the five years period, 43.4% of all cases were positive for alprazolam, followed with 10.0% of nordiazepam and 8.1% of cases with lorazepam. However, the detection of alprazolam decreased in recent year, dropping from 51% out of all drugs in 2017 to 25% in 2019. This may caused by the increase of designer benzodiazepines; such as flualprazolam, flubromazolam, etizolam and others. Screening for flualprazolam began in fall of 2018. In 2019 there were 120 flualprazolam cases, and 73 in the first three months of 2020. Zolpidem continues to be the most frequently seen non-benzodiazepine sedative drug follow by diphenhydramine, doxylamine and hydroxyzine. Most frequently seen drugs in combination with benzodiazepines are THC (62% of all cases) and methamphetamine (18% of all cases) in Orange County, CA.

Conclusion/Discussion:

An overview of benzodiazepines and sedative drugs can provide a perspective on current drug trends that are necessary to monitor. From 2015 to 2019, alprazolam, nordiazepam, and lorazepam continue to be the most prevalence drugs in DUID, death investigation and sexual assault cases. Based on the review of 2019 data, the decrease in alprazolam detection with the increase of flualprazolam, showed a transition from traditional benzodiazepines to new synthetic compounds. Sedative drugs like diphenhydramine, doxylamine, and hydroxyzine had more positive detections in death investigation cases and will continue to be monitored in the coming year for drug prevalence study.

Cannabinoids in Blood and Brain Tissue

Authors and Affiliations: Vanessa Meneses and Melanie Hernandez*, Orange County Crime Lab, Santa Ana, California

Background/Introduction:

Cannabinoids in postmortem cases although widely studied, has presented challenges in the correlation of blood and brain tissue concentrations. Due to their lipophilic nature, postmortem redistribution (PMR) may occur where central blood concentrations may be higher than in peripheral blood. Brain tissue has been hypothesized to retain THC therefore comparing matrices can add to the limited data available on PMR.

Objectives:

The objective of this research was to quantitate Δ-9-tetrahydrocannabinol (THC), 11-hydroxy-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH) in blood and brain tissue samples in order to compare for distribution patterns between the matrices.

Methods:

43 THC positive death investigation cases were selected for this study and divided into two groups. 21 cases were related to a driver death and the other 22 had a non-driving cause of death. When available, the central blood, peripheral blood and brain tissue were analyzed. Brain samples were stored at -20°C while blood samples were stored at 2-4°C in 2% sodium fluoride anticoagulant preservative. Brain tissue was homogenized at 2x dilution with deionized water then sampled at 0.6 g on an analytical balance while blood was sampled at 150 µL. 0.1% formic acid buffer and 25 µL (50 µL brain) deuterated internal standard of each analyte was added to each sample then mixed. Samples were added to Biotage ISOLUTE SLE+ columns then eluted with 30:70 hexane:ethyl acetate. Samples were dried under heated air flow then reconstituted with mobile phase. The analysis was performed on Waters Aquity UPLC using an HSS T3 1.8 µm, 2.1 x 50 mm column. The LC method utilized a 5-minute gradient collecting MRM data with Waters XeVo-TQS in ESI+ mode with two ion transitions. Mobile phases were 100% Acetonitrile and 100% ultra-pure water each with 0.1% formic acid. The limit of quantitation and limit of detections for the LC method were administratively set at 5 ng/mL for THC-COOH and 1 ng/mL for the other analytes.

Results:

Ratio comparison of drivers (non-drivers) for THC, 11-OH-THC and THC-COOH. Central blood (C), peripheral blood (P) and brain tissue (B). Total signifies the total samples with positive results in both matrices compared. THC was detected in the brain in at least half of the cases when also present in central blood along with 11-OH-THC. 11-OH-THC was detected in the brain in higher concentrations than in central blood for all drivers.

Conclusion/Discussion:

Although there is great variability in the data, it is relevant to understanding patterns of distribution. Possible explanations could be the due to drug storage in tissues and unspecified brain regions analyzed. The history of recent use is a factor between matrices. Studies found PMR to increase as postmortem interval increases, but this was not observed possibly due to the time recorded in days than hours/minutes. 11-OH-THC detection in the brain at higher concentrations would be of future interest.

Cannabinoids in Oral Fluid: Limiting Potential Sources of CBD conversion to THC

Authors and Affiliations: Cynthia Coulter*, Margaux Garnier, Christine Moore; Immunalysis Corporation, Pomona, CA

Background/Introduction:

Background: In late 2019, the National Laboratory Certification Program (NLCP) published an article reporting on the potential analytical conversion of 7-carboxy-cannabidiol (CBDA) to 11-nor-9-carboxy-Δ9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) in urine samples. To date, cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC) are both still considered schedule 1 controlled substances. With the recent rise in states legalizing the use of THC and wide availability of products containing containing THC <0.3%, there is a risk for analysts to incorrectly identify whether the donor was using CBD or THC. Previously published research shows about 2% conversion of CBD parent to THC in an acidic SPE extraction (Karschner et al., 2010) If CBDA converts to THC-COOH in urine assays, then potentially CBD may convert to THC in an oral fluid analytical procedure causing the same issues. Most immunoassays on the market do not detect CBD or CBDA in the cannabinoid screening profiles. The risk for false positives in initial screening tests created by intake of high levels of pure CBD would be very low; however, some testing laboratories include in their protocols to confirm all samples regardless of initial screening result, which presents a potential for discrepant results depending on the sample preparation for cannabinoid analysis. Testing programs where screening results are not used could also be a potential risk.

Objectives:

Objective: To determine whether different sample preparation procedures used in oral fluid analysis cause conversion of CBD to THC.

Methods:

Methods: Oral fluid samples were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) on a cannabinoid panel including Δ9-THC, Δ8-THC, THC-COOH, CBD, CBDA, and cannabinol (CBN). Oral fluid samples were initially extracted using a previously published method that includes a base hydrolysis, solid-phase extraction, and derivatization. The degree of conversion of CBD to THC in vitro was measured. The method was then modified to determine which analytical conditions did not convert CBD to THC during the extraction process. No changes were made to the base hydrolysis, derivatization, or instrumentation settings of the published method. Table 1 outlines specific changes to the extraction method. Table 1: Extraction method comparison

Results:

Results: Six oral fluid samples were extracted with the originally published oral fluid method and showed conversion of CBD to Δ9-THC and Δ8-THC. Samples were extracted under the neutral method conditions and showed no conversion of CBD to Δ9-THC and Δ8-THC.

Conclusion/Discussion:

Conclusion: Adjusting the pH of the sample preparation and extraction from pH 2.0 – 3.0 to pH 4.0 - 5.0 can reduce CBD conversion from about 5% to 1%. Removing any acid component to the preparation and extraction procedure can eliminate the conversion all together. Discussion: Adding a single analyte control of CBD at 100 ng/mL to each batch helped to monitor any conversion of CBD to THC during the sample preparation process.

Table 1: Extraction method comparison

	Published Method	**Acid / Neutral Method**	**Neutral Method**
Sample prep	Base hydrolysis	Base hydrolysis	Base hydrolysis; 2 mL water
Column	Trace N	Trace N	Cerex Polycrom THC
Column Conditioning	100 µL glacial acetic acid pH 2.0	100 µL 0.1M acetic acid pH 4.0	No conditioning
Column Wash	1 mL glacial acetic acid: water (80:20)	No acid wash	1 mL water: acetonitrile: ammonium hydroxide (90:10:1)
Eluant	1 mL Hexane: glacial acetic acid (98:2)	1 mL Hexane: glacial acetic acid (98:2)	2 mL Ethyl acetate

Results:

Sample ID	**ELISA Screen Results**	**Original Results (ng/mL)**			**Samples extracted using Neutral Method (ng/mL)**		
		CBD	**Δ9-THC**	**Δ8-THC**	**CBD**	**Δ9-THC**	**Δ8-THC**

1	NEG	399	8	2	387	0	0
2	NEG	127	2	1	90	0	0
3	NEG	171	3	0	144	0	0
4	NEG	483	4	1	481	0	0
5	NEG	90	2	4	96	0	0
6	NEG	132	3	2	90	0	0

Comparison of Sample Preparation Approaches for the Extraction of 11-nor-9-carboxy-Δ9-THC from Urine prior to GC/MS Analysis

Authors and Affiliations: Rhys Jones, Katie-Jo Teehan, Adam Senior, Alan Edgington, Lee Williams, Geoff Davies, Helen Lodder, Steve Jordan, Claire Desbrow & Paul Roberts. Biotage GB Limited, Distribution Way, Dyffryn Business Park, Cardiff, CF82 7TS, UK.

Background/Introduction:

GC/MS is still a mainstay in forensic analysis for drugs of abuse testing in urine. Historically silica-based solid phase extraction (SPE) columns have been used for these target analytes, the exact choice being dependent on drug functionality. The primary urinary target to prove cannabis using is the metabolite 11-nor-9-carboxy-Δ9-tetrahydrocannabinol. Clean up for this analyte prior to GC/MS analysis has traditionally been performed using silica-based mixed-mode anion exchange SPE columns. This poster aims to compare various sample preparation techniques for this analysis. We will aim to compare technique simplicity, streamlined workflow advantages and overall method performance using silica-based mixed-mode SPE, polymer-based SPE, both mixed-mode and reversed phase and supported liquid extraction (SLE).

Objectives:

This poster aims to compare sample preparation techniques demonstrating simplified workflows for the analysis of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol from urine.

Methods:

GC-MS analysis of THC-COOH was performed using an Agilent 7890A GC coupled to a 5975C MSD equipped with a Quickswap device. Chromatography was performed on a Restek Rxi®-5ms capillary column; 30 m x 0.25 mm ID x 0.25 µm using 1.2 mL/min helium flow. All extracts were derivatized using BSTFA/1% TMCS prior to analysis. Positive ions were acquired using electron ionization operated in selected ion monitoring (SIM) mode. The target analyte and corresponding internal standard was spiked into human urine at various concentrations prior to hydrolysis. Sample preparation strategies compared supported liquid extraction (SLE), silica-based and polymer-based SPE, both reversed phase and mixed mode. Extraction strategies focused on overall method performance along with technique simplicity.

Results:

Negative urine was provided by healthy human donors and spiked with 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH) and its deuterated D6 analogue as internal standard. Widely accepted base hydrolysis was performed using 10N NaOH heated at 60 °C for 25 minutes. Complete hydrolysis was assumed but not investigated during these experiments. Post-hydrolysis pre-treatment was investigated and optimized for each sample preparation technique. SLE required acidification prior to extraction due to the presence of the carboxylic acid group. Minimal recoveries without acidification increase to > 80% with corresponding RSDs below 10% using low volume pH control. Extraction solvent investigation illustrated MTBE or hexane-EtOAc combinations to provide improved extraction. Silica-based mixed-mode SPE required acidification prior to loading while polymer-based mixed-mode and reversed phase options didn't due to the more retentive nature of the sorbents. All SPE resulted in recoveries greater than 80% and corresponding RSDs below 10%. Workflow advantages were possible using SLE with a load-wait-elute processing procedure. No washing steps also resulted in elimination of waste disposal and faster overall processing time. Moving from silica-based to polymer based SPE allowed a number of workflow advantages: reduction of bed size for extraction, lower wash volumes and associated waste, elution solvent reduction allowing direct elution into GC vials, elimination of conditioning and equilibration steps (3 in total) and overall processing time. Calibration curves were constructed using blank urine spiked between 1-100 ng/mL. Results demonstrated good linearity and coefficients of determination (r2) greater than 0.99 for all preparation techniques. LOQ determination demonstrated 5-10 ng/mL for SLE using 1 mL of urine, SPE both reversed phase and mixed-mode (silica and polymer based) to be 1-2 ng/mL using 2 mL of urine.

Conclusion/Discussion:

Workflow advantages and associated costs from solvent usage, disposal and time were realised when using polymer-based SPE or SLE compared to traditional silica-based options which offset up front cost differences observed between column technologies. All techniques provided viable options for the extraction of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol from urine prior to GC/MS analysis.

CONFIDENT FENTANYL SCREENING IN URINE BY A HRAM ORBITRAP MASS SPECTROMETER

Authors and Affiliations: Stephanie Samra*, Kristine Van Natta, Xiaolei Xie; Thermo Fisher Scientific, San Jose, CA.

Background/Introduction:

The United States is facing an opioid crisis that includes not only the abuse of prescription drugs but also synthetic opioids like fentanyl. According to the Centers for Disease Control and Prevention (CDC), rates of overdose deaths involving synthetic opioids other than methadone, but including fentanyl, increased 10 % from 2017 to 2018. Over 31,000 people died from overdoses involving synthetic opioids, other than methadone, in 2018. As synthetic opioid and fentanyl use continues to skyrocket so does the need for a fast, accurate method for the simultaneous analysis of fentanyl and its analogues.

Objectives:

Demonstrate the screening capability of a modified Thermo Scientific™ Orbitrap Exploris™ mass spectrometer for fast accurate analysis of fentanyl and its analogues.

Methods:

A liquid-chromatography-high resolution mass spectrometry method was developed for the separation and screening of over 150 fentanyl compounds purchased as a kit from Cayman Chemical™ (Ann Arbor, MI, Fentanyl Analog Screening Kit). Standards were resuspended in methanol and spiked into blank urine matrix and diluted at 9 different concentrations ranging from 0.25 to 100 ng/mL and then diluted 20-fold in water prior to analysis. The chromatographic method used for separation was a 15.5-minute gradient run at 0.5 mL a minute on a Thermo Scientific™ Vanquish Flex™ system consisting of a binary pump, a column oven, and a temperature controlled autosampler. The separation was performed on a Thermo Scientific™ Accucore Phenyl Hexyl column (100 x 2.1 mm, 2.6 µm) maintained at 40 °C. Mobile phases consisted of ammonium formate, 0.1 % formic acid in water for phase A and a mixture of ammonium formate in acetonitrile, methanol, and water (50/50/0.1 v/v/v), 0.1 % formic acid for phase B. Fentanyl analogues were detected on a modified Thermo Scientific™ Orbitrap Exploris™ HRAM mass spectrometer. The mass spectrometer was equipped with a Thermo Scientific™ OptaMax NG ion source with a heated electrospray ionization probe operated in positive and negative switching mode. The orbitrap mass spectrometer was operated in Full MS mode with data dependent MSMS collected for confirmation. Full MS was collected for a scan range of m/z 70 to 1,000 at resolution 35,000 with an AGC target of 1e6 and 100 ms injection time. Data dependent MSMS were collected conditionally for ions above an intensity threshold of 1.5e5 with the loop count set to Top 4, dynamic exclusion set to three seconds, and HCD stepped collision energy set to 17.5, 35, and 52.5. MSMS spectra from the known purchased standards from Cayman Chemical were manually curated and stored in a local MSMS spectral library using the Thermo Scientific™ mzVault™. The retention times of each fentanyl analogue was recorded and was method specific. Urine samples from donors with no known opioid use (3 samples, 5 replicates each) were analyzed on three different days for inter- and intra-day reproducibility using the same analytical method. Following analysis, the data was processed using Thermo Scientific™ TraceFinder™ 5.0 where fentanyl and its analogues were confirmed with expected retention times and the mzVault™ fentanyl analogue MSMS library.

Results:

All 150 fentanyl analogues spiked into the urine samples at 1 ng/mL or greater were confirmed in TraceFinder 5.0 with a confidence of 95% or better across all three days of analysis. Fentanyl analogues had reproducible retention times within 0.2 min retention time when the same Vanquish™ Flex system setup is used on each of the three days. Accurate mass for fentanyl and its analogues was held to less than 5 ppm mass accuracy for all experiments.

Conclusion/Discussion:

A fast and accurate method for the simultaneous analysis of fentanyl and its analogues was created successfully on a modified Thermo Scientific™ Orbitrap Exploris™ HRAM. Retention times and data quality of MSMS spectra collected were reproducible and overall sensitivity and speed of the instrument allowed for the collection of high-quality spectra needed for confident screening.

Death Attributed to Dichlorvos (DDVP) Intoxication

Authors and Affiliations: Robert Almeida*, Priya Banerjee, MD, Laurie Ogilvie, Ewa King, Ph.D., Regina Coffey. Rhode Island Department of Health, Forensic Toxicology, Providence, RI

Background/Introduction:

In November 2019, a deceased black male in his mid-50's was found in a parked vehicle. Scene investigation showed, an empty bottle of brandy, two bottles of "Sniper" pesticide and a suicide note taped to the steering wheel. Both bottles of "Sniper" were open with one empty and one apparently full listing DDVP as the active ingredient. Dichlorvos, also known as DDVP (2,2-dichlorovinyl dimethyl phosphate), is an organophosphate cholinesterase inhibitor commercially available as a pesticide since 1965. Poisonings in humans have occurred through accidental exposure or inhalation and orally by the consumption of contaminated food or direct ingestion (often a suicide method seen in Africa). Exposure causes acute toxicity through inactivation of cholinesterase inducing headache, dizziness, malaise, nausea, miosis, and muscle weakness. Prolonged and acute exposure may lead to death, genotoxic, neurological, reproductive, carcinogenic, immunological, hepatic, renal, respiratory, metabolic, dermal and other systemic effects.

Objectives:

To identify and quantitate concentrations of dichlorvos in various postmortem biological matrices to aid the RI Office of the State Medical Examiner in determining cause and manner of death.

Methods:

Two milliliters of specimens and preparations of tissue/gastric were extracted using an N-butyl chloride method for basic drugs. The samples were analyzed with an Agilent 7693 Autosampler, 7890B Gas Chromatograph and 5977A MSD equipped with an Agilent HP-5MS 5% Phenyl Methyl Silox capillary column, 30m x 250µm I.D. x 0.25µm film thickness. A volume of 1µL was subjected to the injection port with a temperature of 250°C at 9.2 psi and total flow rate of 54 mL/min using helium as carrier gas. Flow through the column was 1mL/min at 9.2 psi and temperature program as follows: initial temperature of 80°C was maintained for 2 minutes, then increased to 180°C over 6 minutes and held for 1 minute. The temperature was then ramped to 275°C over 6.3 minutes and held for 3 minutes for a total run time of 16.3 minutes. The MSD transfer line was 280°C with MS source and Quad at 230°C and 150°C, respectively. The ion source was operated in EI with a fixed electron energy of 70eV. Acquisition was accomplished using SIM monitoring 185>109>79>220 for dichlorvos and 191>115>83>226 for the isotopically labeled deuterated internal standard dichlorvos-D6. Data was analyzed using Agilent's Enhanced Chemstation.

Results:

An autopsy was performed with cardiac blood, femoral blood, vitreous humor, bile, urine, liver, and complete gastric contents all acquired for routine toxicological analysis. A 15-panel drug screen employing ELISA yielded positive findings for cannabinoids and negative results for acetaminophen, cocaine, methadone, opiates, oxycodone, salicylates, tricyclic antidepressants, zolpidem, amphetamine, barbiturates, benzodiazepines, carisoprodol, fentanyl and methamphetamine. Preliminary screening results for ethanol were positive using an alcohol dehydrogenase-based assay. Volatiles analysis using headspace gas chromatography yielded results of 84 mg/dL of ethanol in femoral blood and 87 mg/dL ethanol in the vitreous humor. No further testing was performed to confirm cannabinoids. Table 1. Displays quantitative results obtained for dichlorvos in various postmortem specimens.

Conclusion/Discussion:

Dichlorvos was detected in various postmortem specimens analyzed. No other reportable drugs were detected by GC/MS analysis. Red top tubes were sampled to prevent preservatives from rapidly degrading analyte. Blood esterase activity potentially degraded dichlorvos in cardiac and femoral specimens as these were sampled 85 days after storage at 4°C. Low vitreous humor volume precluded analysis in this matrix.

Detection of Fentanyl Analogs and Novel Synthetic Opioids (NSO) in Hair

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Background/Introduction:

The recent outbreak of novel synthetic opioids (NSOs) into the recreational drug market has been a major contributor to the ongoing opioid crisis. These substances are gaining popularity as substitutes to controlled opioids and are often used as cutting agents or adulterants to heroin and other commonly abused drugs. As a result, timely, and comprehensive drug screening approaches are critically needed to enable forensic laboratories to rapidly and accurately identify these emerging novel substances.

Objectives:

The objective of this study is to develop a comprehensive workflow combining the use of the X500R QTOF System with a simple extraction procedure for specific and sensitive detection of fentanyl analogs and synthetic opioids in hair. The acquisition method generated comprehensive high-resolution MS/MS spectra of all detectable compounds present in the hair matrix, creating a digital record of the sample which enabled accurate identification of the NSO through spectral library matching.

Methods:

Control head hair samples were thoroughly washed with dichloromethane and methanol, dried then grinded prior to being spiked with a mixture of 15 target compounds comprised of fentanyl analogs and NSO. 1 mL of HPLC grade methanol was added to the hair mixture and the samples were incubated at 55˚C for 15 hours without stirring. Following the incubation step, the organic phase was collected in a UHPLC sample vial. Analytes were chromatographically separated at 45°C using a Phenomenex C18 (1.7µm, 2.1 x 100mm) column. Mass spectrometric detection was conducted on a X500R. Samples were evaluated against four main confidence criteria weighted as follows: mass error (15%), retention time (30%), isotope ratio difference (5%), and library score (50%) for all compounds.

Results:

Control head hair samples spiked with all 15 target compounds were injected to build a data processing method. A validation study was performed to determine the linearity, LODs, LOQs, inter- and intra-assay precision and accuracy of the developed workflow. The resulting calibration curves demonstrated excellent correlation of the generated regression curves with with R2 values greater than 0.99 for all 15 target analytes. Inter-day and intra-day precision and accuracy were found to be below 25% and 20%, respectively. The assay showed great reproducibility for concentrations ranging over three orders of magnitude, proving the robustness of the overall workflow. Moreover, limits of detection in matrix (LOD) were found to be in the sub pg/mg range for most of the target analytes used in this study. Real head hair samples from subjects who reported any past-year non-medical opioid use were examined and a number of targeted compounds were successfully identified and quantified using the described workflow. Fentanyl, acetyl fentanyl and furanylfentanyl were positively identified in one of the case samples at concentration of 105.1, 0.9688, and 3.792 pg/mL pg/mg, respectively. The library fit scores (>99.0%) and the combined scores (>90%) providing excellent confidence for the definitive detection of these NSOs.

Conclusion/Discussion:

In this study, a comprehensive workflow for the detection of fentanyl analogs and synthetic opioids in hair was successfully developed using the SCIEX X500R QTOF System. The described method enabled sensitive detection of NSO in real head hair samples at sub pg/mg detection limits. SWATH Acquisition generated comprehensive high resolution MS/MS spectra which enabled accurate identification of the NSOs through spectral library matching. The development of these comprehensive screening methods will provide law enforcement agencies and health professionals a clearer picture of the long term use of these drugs and their evolution in the consumer market as well as consumption trends in specific population.

Disposition of hydrocodone and metabolites in human urine

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Background/Introduction:

Hydrocodone [HC] (Vicodin®), a semi-synthetic derivative of codeine, is used as an antitussive and analgesic medication. It undergoes cytochrome P450 [CYP] dependent O-and N- demethylation to form the metabolites hydromorphone [HM] by CYP 2D6 and norhydrocodone [NHC] by CYP3A4. By comparing with Clinician's prescription, measurement of these compounds is clinically useful to assist in the evaluation of adherence to treatment or to detect misuse.

Objectives:

This retrospective study was performed to evaluate urine concentrations of HC and metabolites from patients in New England, United States. The objective of the study was to identify the parameters that best reflected adherence to treatment and assess the impact of gender and age on metabolite formation.

Methods:

De-identified consecutive random urine specimens from 2019 were subject to identification and quantitation of total HC, HM, and NHC by liquid chromatography tandem mass spectrometry [LC-MS/MS]. Using deuterated internal standards, specimens were prepared by solid phase extraction with a SPEware/Tecan ALD II automated liquid dispenser into 1mL 96 deep well plates [Thermo]. Eight calibrators [20-10,000 ng/mL, Cerilliant] produced a quadratic curve fit with 1/x2 weighting. The lower reporting limit was 50 ng/mL. Quality control samples included drug-free urine, and 62.5, 1000, 8000 ng/mL and a codeine-6-beta-D-glucuronide hydrolysis control at 15,000 ng/mL. Analysis was performed with a Shimadzu LC20AD/XR pump and MPX autosampler and Sciex 4500 MS with a Phenomenex Kinetex Biphenyl, 50 x 3.0 mm, 2.6 mcm, 100Å column. Analyte resolution was achieved with a gradient binary mobile phase consisting of an aqueous phase of LCMS grade water and 0.1% formic acid, and an organic phase of LCMS grade methanol and 0.1% formic acid.

Results:

A total of 4410 specimens were positive for at least one analyte (Table 1). [ng/mL]. All three analytes were detected in 845 [19.2%] specimens; HC+NHC combination was found in 295[6.7%]; HC+HM combination was found in 31[0.7%]; and HM+NHC combination was found in 44[1.0%] of specimens. HC was detected as a single drug in 27 [0.6%] specimens and NHC alone in 107 [2.4%]. HM alone was detected in 3061 [69.4%] specimens. It is unknown how many of these may have been from HM ingestion. Calculation of metabolite ratios [N=845] revealed the mean and median for HC/HM [5.1±7.6, 2.9] was 4-6 fold higher than that of HC/NHC [0.8±0.7, 0.7]. In order to assess the influence of gender and age on metabolite formation, specimens containing HC, NHC and HM were subdivided as shown in Table 2. This subset of 842 samples included 440 [52.3%] females [3 patients were excluded due to missing gender information].

Conclusion/Discussion:

In this retrospective study of HC and metabolites in urine we found that all analytes were detected throughout the analytical measurement range. NHC concentrations were of a similar magnitude as HC as reflected in the metabolic ratios, HC/NHC being closer to 1 [0.8] than HC/HM [5.1]. The metabolite NHC was also more likely than parent drug to be detected alone.

Analysis of Drugs on Impregnated Prison Letter Samples Using LC/Q-TOF-MS

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Background/Introduction:

Novel psychoactive substances (NPS), are recreational drugs that provide the effects of classic drugs like cannabis, cocaine and amfetamine. The use of NPS in UK prisons is regarded as endemic, and a problem, increasing violence, aggression and disruptive behaviour.

Objectives:

The current research was conducted in order to identify the variety of emerging novel psychoactive substances impregnated onto letter samples sent to prison inmates. The aim was to help rapid detection and definite identification, enabling the scope of the problem to be established.

Methods:

Suspected drug-impregnated letters were detected by Her Majesty's Prison Service staff using NPS-trained search dogs or by a drug 'Itemiser' for some drugs and Spice (Rapiscan®, 2020). From each piece of paper, approximately a one square cm of paper (especially from stained areas) was cut from different locations and placed into separate 1.5mL Eppendorf tubes with 1mL of 50% (v/v) methanol in LC-MS-grade water. Extracts were prepared from the letters by vortex-mixing (30min). They were screened for NPS and other compounds using an Agilent Technologies 1290 Infinity II – 6545 Q-TOF LC/MS instrument with electrospray ionization in positive ion mode. Drug separation was performed over a total of 13 min using a simple linear gradient of water (A), and methanol (B), both contained 0.01 % (v/v) formic acid and 5 mmol/L ammonium formate at a flow rate of 400 µL/min. The gradient profile was: 5% B in 0–1 min and 12 min, 100% B in 10–12 min. Sample injection volume was 2µL.

Results:

A drug database which consists of more than 200 samples was created and used it for the identification of unknown substances usually seen in forensic laboratories. 332 samples collected in 2019 from seven UK prisons were screened with this method. In 96% of all samples, nicotine, abused prescription drugs, other medication and Synthetic Cannabinoid Receptor Agonists (SCRAs), were present. UK Class A drugs (alfentanil, cocaine, heroin, 3, 4-methylenedioxymethamfetamine (MDMA, 'ecstasy'), morphine etc.) and UK Class B drugs (amphetamines, codeine, ketamine etc.) were also detected. Synthetic cannabinoids were the most common drug category detected in prison letter samples with 51% of all samples.

Conclusion/Discussion:

This research is a confirmation of the findings of the only other publication in this area (Ford and Berg, 2018) and confirms that NPS being brought into UK prisons via drug impregnated letters posted to prisoners still remains a problem. With analytical confirmation of drug impregnated letters sent to prisoners, which including NPSs, we have generated qualitative data to assist detection and identification information and have demonstrated that novel psychoactive substances are still entering UK prisons in this way.

Efficacy of Sample Preparation Techniques on Whole Blood Prior to High-Resolution Mass Spectrometry (HRMS) Drug Screening

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Background/Introduction:

The shift from immunoassay to mass spectrometry-based toxicological screening presents a number of challenges for operational laboratories. Sample preparation is just one of those challenges, because electrospray ionization (ESI) techniques are susceptible to matrix effects that can negatively impact assay performance. During toxicological screening, fast, efficient extraction protocols are vital. Supported liquid extraction (SLE) is an alternative to conventional solid phase or liquid/liquid extraction (LLE) techniques. In this study, liquid chromatography-quadrupole/time-of-flight mass spectrometry (LC-Q/TOF MS) was used for toxicological screening of whole blood specimens. SLE in combination with protein precipitation and lipid removal were evaluated for drugs of interest, including Tier I and select Tier II drugs of abuse . Logan, BK, D'Orazio, AL, Mohr, ALA, Limoges, JF, Miles, AK, Scarneo, CE, Kerrigan, S, Liddicoat, LJ, Scott, KS and Huestis, MA (2017) Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities-2017 Update. Journal of Analytical Toxicology, 42(2), 63-68.

Objectives:

To determine the utility of sample clean-up steps, including protein precipitation and lipid removal devices prior to supported liquid extraction.

Methods:

Tier I drugs at the recommended cutoffs and other selected compounds from representative categories within Tier II were fortified into blood. Prior to SLE, blood (0.5 mL) was subjected to i) no pre-sample clean-up (control), ii) protein precipitation (PPT), or iii) lipid removal (Agilent Captiva EMR-Lipid) using replicate analysis. Three solvents were selected for PPT (acetone, acetonitrile and methanol). Lipid removal devices were utilized with and without the use of 1% formic acid. Following completion of the sample pre-treatment step, 500 µL of 0.1% formic acid in water was added and 750 µL of sample was extracted using a 1 mL SLE column (Biotage Isolute SLE+). After sample adsorption to the SLE sorbent, drugs were eluted with varying amounts of 90:10 dichloromethane/isopropanol and methyl-tert-butyl ether (MTBE). The extracts were then fortified with acidic methanol and evaporated to dryness under nitrogen at 50°C. Extracts were reconstituted in 20 µL of a 60:40 mixture of mobile phase A:B (5mM ammonium formate with 0.01% formic acid in water/0.01% formic acid in methanol). After centrifugation, the supernatant was transferred to a clean autosampler vial and analyzed in positive and negative ESI using All Ions acquisition on an Agilent 6530 LC-Q/TOF-MS.

Results:

PPT exhibited the greatest variability in analyte abundance for the acidic drugs (analyzed in negative ESI) when normalized to the control group. Analyte loss following SLE pre-treatment was particularly evident for 11-nor-9-carboxy-delta-9-THC (THCA) and valproic acid (VPA). Significant loss (90%) of VPA was observed following use of the lipid removal device. Optimum results for THCA were achieved using SLE with no additional pre-treatment. Basic and neutral drugs exhibited good reproducibility and abundance using the majority of conditions tested. However, buprenorphine results were inconsistent following use of the lipid removal device.

Conclusion/Discussion:

Although additional sample pre-treatment exhibited improved performance for some basic and neutral drugs, SLE alone provided satisfactory performance for the majority of drugs. However, overall robustness of the method requires further investigation.

Evaluation and Applicability of Rapid Point-of-Care Screening of Postmortem Urine at Autopsy

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Background/Introduction:

The US is observing higher rates of drug-related deaths than previously recorded. Subsequently, mechanisms to process these cases, and other death investigations, at a faster rate are beneficial. A quick and straightforward method of drug screening carried out by the forensic pathologist during the autopsy may be warranted if immediate results are required to triage certain deaths, or if the forensic toxicology report is often delayed significantly. This may be more so utilized in jurisdictions without in-house toxicology facilities.

Objectives:

To evaluate the point-of-care (POC) screening device, Alere iCup DX 14, for the screening of 14 classes of common drugs of abuse in postmortem urine, in the context of a forensic pathologist performing the autopsy-side test for possible case triage.

Methods:

One hundred-ninety postmortem urine samples from the Office of the Chief Medical Examiner of the City and County of San Francisco from November 2018 through June 2019 were analyzed via the iCup device at autopsy for the presence of amphetamines, barbiturates, benzodiazepines, buprenorphine, cocaine, 3,4-methylenedioxymethamphetamine (MDMA), methamphetamine, methadone, morphine, oxycodone, phencyclidine, propoxyphene, tricyclic antidepressants, and tetrahydrocannabinol (THC). Screening with the iCup required 10 mL of urine, with preliminary positive and negative results obtained within 10 min. Positive and negative results obtained from the screening kit were evaluated against confirmatory analysis results obtained using routine forensic toxicology analyses, typically employing LC-MS/MS methodologies, for the detection of a combination of over 85 different target drugs and metabolites by the forensic toxicology laboratory.

Results:

Sensitivity for each respective iCup drug class ranged from 66.7% (buprenorphine) to 100% (methadone, tricyclic antidepressants), with an average of 90.9% and a median of 92.2%. Specificity for each respective iCup drug class ranged from 90% (benzodiazepines) to 100% (amphetamines, barbiturates, buprenorphine, MDMA, methadone), with an average of 98.3% and median of 99.3%. No iCup false positives were observed for amphetamines, barbiturates, buprenorphine, MDMA, and methadone. There were an average of two false positives cases (range 1-4) for cocaine, methamphetamine, morphine, oxycodone, tricyclic antidepressants, and THC. Benzodiazepines yielded the highest false positive rate at 17 cases. No false negatives were observed for barbiturates, methadone, and tricyclic antidepressants. False negatives ranged from 1-6 cases with an average of three for amphetamines, benzodiazepines, buprenorphine, cocaine, MDMA, methamphetamine, morphine, oxycodone, and THC, with morphine yielding the highest false negative number of cases at six.

Conclusion/Discussion:

This applicability study in authentic postmortem samples provides an evaluation of the performance of the iCup in forensic casework when utilized by a forensic pathologist at autopsy compared to traditional routine forensic toxicology analyses. Overall performance was of similar quality to that indicated by the manufacturer via previous study confirmed by GC-MS. In conjunction with screening for other commonly used drugs, the Alere iCup DX 14 may be useful in giving forensic pathologists direction in triaging casework when understood and interpreted appropriately. This may prove useful in scenarios where an unsuspected positive finding was observed in a non-drug related natural or suicide case where toxicology testing may have likely not been performed due to cost of testing, accessibility and/or forensic facility protocols.

Evaluation of a Model for Providing Cost Effective, Accessible Continuing Education Content to Forensic Toxicologists

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Background/Introduction:

Adequate training and continuing education are critical to ensuring the quality and credibility of forensic science practice (Strengthening Forensic Science in the United States: A Path Forward, National Academy of Sciences, p. 29). Certification programs such as the one administered by the American Board of Forensic Toxicology (ABFT) have been applauded and recognized for their rigor in establishing educational, training, and experience requirements for forensic science professionals. A gap exists, however, in an individual's ability to identify cost effective, accessible continuing education content to meet these certification requirements. Specifically, there is no programmatic approach to educational needs assessment & content development, lack of an optimized delivery channel (face

to face, online, hybrid) and no central repository of available educational opportunities. As a result, not all forensic toxicologists are getting access to the educational content required to remain current in their field.

Objectives:

Online continuing education is on the rise as this modality of dissemination allows for accessibility, convenience and affordability. This study presents a detailed model and its subsequent assessment for the development, optimized delivery and archival of compelling, cost-effective online continuing education content for the forensic science community.

Methods:

The model was tested through the launch of an online symposium series, offered free of charge to participants. Each day representing a virtual Master Class - an in-depth examination of a topic presented by world-class practitioners - having a unique mastery of the subject. A three-year study of over 2,000 participants representing > 60 countries was undertaken to assess and improve the efficacy of the model relative to its stated goals.

Results:

Attendees of the symposium were eligible for 0.75 CE credit points per day (3.75 points upon participation in the entire week's program) from the American Board of Forensic Toxicology (ABFT). Participation was tracked via the learning management software with certificates issued to attendees at the conclusion of each day. For the 2020 Current Trends in Forensic Toxicology Symposium, a total of 5,500 daily certificates were issued equating to a total of 4,124 ABFT CE credit points. A number of best practices were identified along with opportunities to further enhance the model to meet the stated objectives of accessible, compelling, cost-effective continuing education for professionals. One such example regards operational excellence by offering multiple levels of attendee technical support including introductory verbal instructions, prominently displayed FAQ troubleshooting documents, automated software driven system compatibility tests, access to live technical support during the event and rehearsed contingency plans for speakers in order to address potential system failures.

Conclusion/Discussion:

This study provides empirical support and best practices for the use of cost effective online continuing education to support to professional development needs of the forensic toxicology community. The results demonstrated that participants found the online symposium model fulfilled expressed learning goals and their engagement in the forum met required continuing education requirements.

Extraction of Chloroquine (CQ) and Hydroxychloroquine (HCQ) from Different Biological Matrices Prior to LC-MS/MS Analysis

Authors and Affiliations: *Mohamed Youssef[1], Stephanie Marin[1], Elena Gairloch[1] 1-Biotage, Charlotte, NC, USA

Background/Introduction:

Hydroxychloroquine and chloroquine are common antimalarial drugs with long half-lives. They are currently being tested as potential treatments for COVID-19. Due to their high publicity in the media, many forensic toxicologists are working to develop methods to extract and detect these compounds in different matrices to understand the adverse effects, potential toxicity, and drug interactions associated with these drugs. These compounds are not typically included in general unknown screens. A simple sample preparation procedure that delivers clean extracts and analyte recovery greater than 90% for CQ and greater than 82% for HCQ with minimum matrix effects and high process efficiency was developed.

Objectives:

The objective of this study was to develop a fast, reliable extraction method for chloroquine (CQ) and hydroxychloroquine (HCQ) from different human matrices (whole blood, serum, and urine) using ISOLUTE® SLE+ Supported Liquid Extraction plates prior to LC-MS/MS analysis

Methods:

A 100 µL aliquot of blank biological fluids (whole blood, serum and urine) were spiked with equal concentrations of 10 ng/mL of both analytes, diluted with 300 µL of 0.5 M ammonium hydroxide, and mixed well. 375µL of the pretreated samples were loaded onto the Biotage ISOLUTE® SLE+ 400µL plate following the proper SLE protocol, then eluted with 750µL of ethyl acetate twice. Extracts were then evaporated under nitrogen using a Biotage® SPEDry at 40°C, reconstituted in 100 µL of mobile phase (A) ACN:20 mM Ammonium formate (15:85, v/v) + 0.2% formic acid and analyzed with Agilent 1260 HPLC coupled with Sciex 4000 triple quadrupole mass spectrometer.

Results:

Matching the initial conditions of the LC gradient allowed for improved chromatographic separation and minimized matrix effects from coeluting peaks from the matrices. Recoveries, matrix effects, and process efficiencies for both analytes were calculated for all three matrices. For all three matrices, calculated matrix effects for chloroquine showed slight enhancement (between 2-12%), while hydroxychloroquine showed slight suppression (between 4-13%). Both analytes had calculated recoveries of 82-97% when using ISOLUTE® SLE+ for all three matrices. Process efficiencies when using this extraction technique were 66-99% for all three matrices.

Conclusion/Discussion:

Extraction of chloroquine and hydroxychloroquine using ISOLUTE® SLE+ and analysis by LC-MS/MS resulted in sensitive, fast, efficient quantitation of these drugs in whole blood, serum and urine. Extraction by SLE using the same conditions for three different matrices simplifies implementation of the method in toxicology laboratories.

Extraction Techniques to Analyze Synthetic Benzodiazepines in Various Biological Matrices

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Background/Introduction:

Synthetic benzodiazepines are some of the most abused novel psychoactive substances seen today. Because of this, it is important for forensic laboratories to develop methods to analyze for these compounds in biological matrices like blood and urine. Several extraction techniques can be used that offer both higher recoveries and reduced matrix effects, depending on the technique, including dual mode extraction, solid phase extraction, and supported liquid extraction. The 12 compound benzodiazepine panel was analyzed using LC-MS/MS and all recoveries and matrix effects were calculated for the various extraction methods.

Objectives:

The objective of this study was to develop extraction methods for a novel benzodiazepine panel and determine recoveries and matrix effects for the various extraction techniques.

Methods:

A 100 µL sample of urine or whole blood was used for each sample preparation technique. Samples were fortified at either 10 ng/mL or 100 ng/mL. For the supported liquid extraction methods (ISOLUTE SLE+, Biotage), samples were pretreated with 100 µL of 1% ammonium hydroxide, loaded onto the extraction plate, and eluted with either dichloromethane, ethyl acetate, MTBE, or 95:5 dichloromethane/isopropanol. For the solid phase extraction method, mixed mode cation exchange was used (EVOLUTE EXPRESS CX, Biotage). Samples were pretreated with 100 µL of 0.1% formic acid and were loaded onto the extraction plate. Columns were washed with water, 0.1% formic acid, and methanol and eluted with either dichloromethane/isopropanol/ammonium hydroxide (78:20:2) or ethyl acetate/acetonitrile/ammonium hydroxide (78:20:2). For dual mode extraction (ISOLUTE HYDRO DME+, Biotage), two sets of samples were loaded onto the extraction plate, 10 µL of formic acid was added to one set of samples, 600 µL of acetonitrile was added to both sample sets, and samples were pushed through the sorbent using positive pressure (PRESSURE+ 96, Biotage). After extraction, the samples were evaporated and reconstituted in a 90:10 mix of 0.1% formic acid in water/0.1% formic acid in methanol. The samples were analyzed using a Shimadzu Nexera X2 UPLC coupled with a Sciex 5500 tandem mass spectrometer. (All benzodiazepine compounds in the panel, as well as their specific mass spectrometer parameters are listed in the attached table.)

Results:

Recoveries and matrix effects for the 12 novel benzodiazepines were calculated for both matrices for each extraction technique. For the urine samples, the ISOLUTE SLE+ methods with MTBE and dichloromethane/isopropanol elution solvents resulted in the highest recoveries (greater than 80%), while the ISOLUTE HYDRO DME+ method without using formic acid resulted in the lowest recoveries for a couple of compounds in the panel (less than 20% for bromazepam and clobazam). The ISOLUTE SLE+ method with a dichloromethane/isopropanol elution solvent resulted in the cleanest extracts (lowest matrix effects) for the urine samples. For the blood samples, the ISOLUTE SLE+ methods with the ethyl acetate and MTBE elution solvents resulted in the highest recoveries (greater than 80%). The lowest recoveries were seen using the ISOLUTE HYDRO DME+ method with formic acid (5-20% recoveries for most compounds). The cleanest extracts were seen with the ISOLUTE SLE+ method with a dichloromethane/isopropanol elution solvent (matrix effects within 30%).

Conclusion/Discussion:

When developing a novel benzodiazepine method, there are multiple extraction techniques that are suitable. The compounds in the panel, desired extract cleanliness, desired recoveries, and extraction time help to determine the optimum methods for both whole blood and urine samples. The ISOLUTE HYDRO DME+ methods had the fastest extraction times, but these methods had increased matrix effects and, for some compounds, lower recoveries. The EVOLUTE EXPRESS CX methods had the longest extraction times, but resulted in cleaner extracts. The ISOLUTE SLE+ methods had a shorter extraction time, cleaner extracts, and higher recoveries. The ISOLUTE SLE+ methods also had reduced solvent waste, especially when compared to the solid phase extraction methods.

MS Parameters for all Novel Benzodiaepine Compounds

Compound	Retention Time	Q1	Q3	Declustering Potential	Collision Energy	CXP
Clobazam	6.74	300.951	259.1	136	31	6
		300.951	224.1	136	45	10
Bromazepam	6.74	317.929	301.0	16	13	8
		317.929	259.0	16	35	16
Phenazepam	6.71	348.917	206.1	21	47	12

		348.917	184.1	21	41	8
Estazolam	6.86	295.000	267.0	96	33	4
		295.000	205.2	96	55	14
Clonazolam	6.57	353.962	308.1	181	37	14
		353.962	280.1	181	49	8
Prazepam	7.73	324.992	271.1	216	33	10
		324.992	140.1	216	47	10
Flubromaze-pam	6.57	334.904	226.1	66	39	12
		334.904	185.9	66	41	12
Etizolam	7.34	342.955	314.1	216	35	6
		342.955	259.1	216	47	8
Delorazepam	6.58	304.946	166.0	121	71	8
		304.946	140.2	121	71	26
Pyrazolam	6.51	355.938	206.2	156	47	12
		355.938	167.2	156	65	16
Diclazepam	7.15	318.927	154.1	141	39	10
		318.927	227.2	141	47	6
Nimetaze-pam	6.94	296.000	250.1	126	35	8
		296.000	221.2	126	47	8

Flualprazolam: The Latest Designer Benzodiazepine to Wreak Havoc

Authors and Affiliations: Melissa Lloyd*, Trista Wright, and Chad Harris, Virginia Department of Forensic Science, Richmond, VA, USA

Background/Introduction:

Benzodiazepines are among the most commonly prescribed drugs in the U.S. Furthermore, there are non-FDA approved benzodiazepines sold on the black market known as designer benzodiazepines. Flualprazolam is classified as a designer benzodiazepine that was first synthesized in the 1970s, but never marketed. Flualprazolam is structurally similar to alprazolam, but pharmacological data suggest it is more potent (Krotulski et al. CFSRE, 2019). The Virginia Department of Forensic Science (VDFS) developed and validated a method for flualprazolam in the summer of 2019 that was added to the existing benzodiazepines quantitation and confirmation method by LCMSMS.

Objectives:

This study aimed to examine case data of positive flualprazolam cases and to identify any trends seen in case history in impaired driving cases. The number of cases, demographics, case history, and other drugs found were examined. This analysis was intended to provide a better understanding of flualprazolam impaired driving cases and identify any trends that may help in mitigating risk by raising public awareness and in designing preventive measures to reduce the use of flualprazolam.

Methods:

A certified reference material for flualprazolam was obtained from Cayman Chemical and Cerilliant. Enzyme-linked immunosorbent assay (ELISA) kits were purchased from Immunalysis and flualprazolam was screened via the benzodiazepine kit at cut-off concentration of 4 ng/mL on the Tecan Evo 75. Flualprazolam did cross react with the benzodiazepine kit and was approved for screening by immunoassay. Flualprazolam was confirmed by liquid chromatography tandem mass spectrometry (LCMSMS) on an Agilent 1260 Infinity II liquid chromatogram with a 6460 tandem mass spectrometer with an electrospray ionization source (verified on Agilent 1260 Infinity LC with a 6430 QQQ too). The limit of detection for flualprazolam was 5 ng/mL. Driving while intoxicated or driving under influence of drug cases, analyzed by VDFS that confirmed positive for flualprazolam above 5 ng/mL were included in this study. The samples were collected by police agencies around the state of Virginia from drivers or suspects as indicated on the Request for Laboratory Examination and/or Certificate of Blood Withdrawal. Demographics including age, sex, and race/ethnicity were evaluated. Overall case results were examined and police reports requested to identify any trends seen in positive flualprazolam cases.

Results:

Flualprazolam, was first identified in impaired driving cases in Virginia in early June 2019. Thus far, 67 impaired driving cases both fatal and non-fatal have been identified in Virginia. The majority of drivers (61%) were male (21% female). The mean (median) age was 27 (24.5) years old with a range of 19 to 55 years old. Approximately 42% of drivers where white and 21% black. The most common drugs/drug classes found in cases with flualprazolam were cannabinoids (37%), opioids (22%), benzodiazepines (16%), cocaine/metabolites (16%), buprenorphine/norbuprenorphine (10%), and amphetamines (6%). Police reports indicate observations of impairment that are consistent with CNS depressants.

Conclusion/Discussion:

In December 2019, The Center for Forensic Science Research and Education released a bulletin identifying flualprazolam as a potent benzodiazepine that had been identified in 44 cases (Krotulski et al. CFSRE, 2019). Additionally, reports from the Georgia Bureau of Investigation identified flualprazolam in 17 cases. GBI reported the most common drug found in conjunction with flualprazolam was cannabinoids (Morrison et al. SOFT, 2019), which is consistent with our findings. The AACC indicated that many cases were designer benzodiazepines have been reported were frequently with other commonly abused drugs (Marin et al. AACC, 2019). The AACC also indicated that many designer benzodiazepines are sold under the name of FDA approved benzodiazepines. There were multiple cases in Virginia were the suspect admitted to taking “Xanax “and no alprazolam was found.

Focused on Death: A Rare Suicidal Overdose of Atomoxetine

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Background/Introduction:

Atomoxetine, sold under the brand name Strattera®, is a non-stimulant medication used to treat Attention Deficit Hyperactivity Disorder (ADHD). Unlike other ADHD medications, atomoxetine is not federally scheduled due to the believed low risk of abuse. The average oral dose for atomoxetine ranges from 10 to 100 mg and produces peak plasma concentrations ranging from 0.1 to 0.6 mg/L in healthy individuals and 0.1 to 2.6 mg/L in those with hepatic impairment. This case study presents a 30-year-old white male found deceased in his residence in the presence of empty prescription bottles. There was a history of illicit drug use and suicidal ideations due to a recent separation from his wife. Medical records indicated prescriptions for both atomoxetine and fluoxetine. At this time, there are limited reported fatalities from atomoxetine toxicity. Reported toxic blood levels of atomoxetine range from 0.1 mg/L to 8.3 mg/L. Fluoxetine (Prozac®) is a selective serotonin reuptake inhibitor commonly used to treat depression. Therapeutic fluoxetine levels generally range from 0.1 to 0.8 mg/L. Although atomoxetine is believed to be of low risk for abuse, studies have shown increased suicidal ideations especially in children and teenagers who are diagnosed with ADHD and taking this medication.

Objectives:

This case study demonstrates novel toxicological findings and implications from a sudden, cardiac death resulting from the acute toxicity of atomoxetine and fluoxetine in a 30-year-old male.

Methods:

TThe samples submitted to the Virginia Department of Forensic Science Western Laboratory included femoral blood, iliac blood, vitreous humor, and urine. The femoral blood sample underwent routine screening following standard operating procedures for volatiles using headspace gas chromatography coupled with flame ionization detection, drug screening using Immunanalysis enzyme linked immunoassay (ELISA), and a basic drug screen using full scan gas chromatography-mass spectrometry (GC-MS). Routine testing in femoral blood for the presence of ethanol and ELISA drugs/drug classes was negative. The basic drug screen in femoral blood identified atomoxetine and fluoxetine. Atomoxetine was confirmed by GC-MS; however, a validated quantitative method was not available. A sample of iliac blood was sent to a referral laboratory for atomoxetine quantitation.

Results:

The results revealed atomoxetine in the iliac blood sample at 21 mg/L, nearly 10 times that of the upper therapeutic levels. Fluoxetine was quantitated at 1.6 mg/L in femoral blood by SPE using gas chromatography coupled with a nitrogen-phosphorous detector. This level of fluoxetine is a potentially fatal level. The autopsy results included a superficial abrasion to the mid-forehead that was consistent with a collapse as well as findings that were typically seen in drug overdoses such a congestion of the face/neck, conjunctival congestion, and pulmonary edema (right lung weight 950 g/left lung weight 780 g). Chalky material was noted in the gastric contents. Natural pathology included hypertensive cardiovascular disease seen as cardiomegaly (heart weight 410 g), arteriolonephrosclerosis, emphysematous lung disease, and macrovesicular steatosis.

Conclusion/Discussion:

This case is among the few literature reports of atomoxetine fatalities with blood concentrations above 20 mg/L. The atomoxetine concentration found in the blood was more than double the amount of toxic levels reported in literature. Given the lethal atomoxetine and fluoxetine blood concentrations, the Medical Examiner ruled the cause of death as acute atomoxetine and fluoxetine toxicity and the manner of death as a suicide.

Forensic Authentication of Hemp-Derived Products Using Mass Spectrometry

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Background/Introduction:

The popularity of CBD products has been on the rise following a recent change in federal legislation lifting the regulatory status of hemp-derived products as Schedule 1 controlled substances. Although hemp extracts and hemp-derived products are now legal on a federal level, many of these commercially-available products contain levels of THC above the federal legal limit. As a result, there is an urgent need to develop new tests capable of accurately detecting and quantifying total THC levels present in alleged hemp-derived products to ensure its concentration is below the federal legal limit.

Objectives:

The objective of this study is to develop a comprehensive workflow for the detection and quantitation of 11 cannabinoids using HPLC-UV in tandem with a SCIEX QTRAP 6500+ system. The presented workflow provides an accurate measure of THC levels in hemp-derived products to ensure its concentration is below the 0.3% by dry weight federal legal limit.

Methods:

A total of 4 hemp-derived samples were tested in this study. They included 3 hemp flower strains and one hemp distillate. Flower samples were homogenized prior to extraction. Distillate samples were processed without homogenization. 0.2 gram of each sample was mixed in 10 mL of acetonitrile. The resulting solution was shaken vigorously and sonicated for 30 minutes. Following sonication, the solution was centrifuged for 5 minutes at 1,600 rpm. The extract was filtered using a 0.2 µm nylon syringe filter. The filtered extract was then diluted 1:100 (v/v) with acetonitrile. Analytes were chromatographically separated at 25°C using a Phenomenex Luna Omega Polar C18 column (3µm, 4.6 x 150mm) column. Mass spectrometric detection was conducted on a QTRAP 6500+ System operating in positive electrospray mode using a multiple reaction monitoring (MRM) algorithm to ensure acquisition of an adequate amount of data points for quantifiable data.

Results:

Hemp-derived samples were prepared using the aforementioned procedures and injected to build a data processing method. Accurate quantification of both high- and low-abundance cannabinoids is challenging due to the wide range of cannabinoid concentrations in cannabis and hemp samples. The dual detection system enabled detection of low-abundance species at the low-end of the potency range using the MS/MS system and the high-abundance species at the high-end of the potency range using the PDA detector. The robustness and reproducibility of the newly developed workflow was investigated by calculating the continuing calibration verifications (CCVs) every 10 samples. The results indicate good method reproducibility of delta-9-THC in a 0.5 ppm MS/MS CCV and a 25 ppm PDA CCV with RSDs of 1.8% and 1.4%, respectively. In addition, the calculated CCV values were within the desired 25% of the expected concentration throughout the course of the run, highlighting the high accuracy of the developed method. Using this comprehensive workflow, accurate quantification of total THC was investigated for four hemp-derived samples. Analysis of a hemp flower showed that the total percentages of THC and CBD were 0.15% and 5.93%, respectively. Out of the four samples analyzed, only one (Mile High Hemp Flower) contained THC levels below the 0.3% by dry weight federal legal limit.

Conclusion/Discussion:

In this study, a comprehensive workflow for the detection and quantitation of hemp-derived products was successfully developed. The combination of HPLC-UV in tandem with a SCIEX QTRAP 6500+ system is shown to provide a substantial advantage to enable accurate cannabinoid quantification across the entire potency range. The versatility of the described workflow allows accurate quantification of cannabinoids without the need for multiple sample injections and dilutions to ensure the total THC concentration in hemp-derived samples fall below the 0.3% by dry weight federal legal limit.

Got Tramadol. Method for the Determination of Tramadol and its Metabolites in Breast Milk.

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Background/Introduction:

Tramadol is an opioid used in the treatment of moderate to moderately severe pain including pain associated with labor and following Caesarian sections. It acts on the mu opioid receptor and is a serotonin-norepinephrine reuptake inhibitor. The major metabolites produced are O- desmethyl tramadol (pharmacologically active) and N-desmethyl tramadol. Tramadol's use during pregnancy is generally avoided and may cause some reversible withdrawal effects in the newborn. Its use during lactation is also advised against, but a small trial found that infants breastfed by mothers taking tramadol were exposed to <3% of the dose, and found no evidence of a harmful effect on the newborn.

Objectives:

To develop and validate a method for the analysis of Tramadol (T) and metabolites, O-Desmethyl tramadol (O-DT), N-Desmethyl tramadol (N-DT) in breast milk and confirm presence/absence in authentic breast milk samples.

Methods:

Tramadol, its metabolites and isotopically labeled standards, were extracted using Oasis® MCX µElution plate and analyzed with a Waters AcQuity Xevo TQD Ultra performance liquid chromatography tandem mass spectrometer (UPLC-MS/MS). Chromatographic separation was performed using a UCT Selectra® DA (5cm x 2.1mm, 3µm) column. The mobile phase consisted of (A) 20mm ammonium formate in water and (B) 20mm ammonium formate in methanol. The gradient used was: 0.0 to 1.5 minutes at 60:40, 1.5 to 3 minutes at 30:70, 3 to 3.5 minutes at 95:5 at 3.6 minutes. The flow rate was 0.6mL/minute and column temperature was 40°C. Acquisition mode was in Multiple Reaction Monitoring (MRM) in positive ion mode, m/z: Tramadol - 264>42, 58; Tramadol-d3 -268>42, 58; O-DT – 250>42, 58, 232, O-DT-d6 – 256>45, 64, 238 and N-DT 250>44, 232). Total runtime was 4.0 minutes. Sample preparation: Due to limited access to genuine breast milk, Enfamil® Premium™ newborn infant formula was used in preparing calibrators and control materials; and, saponification with 1N HCl prior to SPE extraction was used to remove the high lipid content in breast milk. Sample preparation was performed in microcentrifuge tubes, 100µL of sample and 100µL 1N HCl was added to 10µL of ISTDs (10 and 20ng Tramadol-d3 and O-DT-d6, respectively). Samples were vortex mixed, allowed to stand for 5 minutes and centrifuged. The supernatant was transferred to an Oasis® µElution plate conditioned with consecutive 200µL methanol, followed by 200µL water. Tramadol and its metabolites were eluted with 50µL ACN:MeOH:NH4OH (3:2:0.25) mix, diluted with 100µL H20 and 5.0µL injected for analysis. Method Validation: The method was validated using ASB/ANSI 036 guidelines as a basis for linearity, accuracy and precision, stability, carryover and limit of detection studies. Calibrators and controls were prepared in infant formula at 10-1000ng/mL. The lower limit of quantitation (LOQ) was administratively set at 10 ng/mL. Validation controls were prepared at 10, 30, 150, 750, and 5000ng/mL (1:4 dilution control). Accuracy and precision were determined from the prepared QC samples. De-identified authentic breast milk samples (N=19) from the VCU lactation services and the Children's Hospital of Richmond at VCU were analyzed.

Results:

Calibration curves were determined to be linear from 10-1000ng/mL, (r^2=0.9990). The bias of the controls was within the ± 20% range; intra and inter-run precision had CVs ≤ 11%. All analytes were stable under the freeze-thaw cycles and bench top stability conditions. Analytes were stable for72 hours post preparation, No carry-over was observed in the negative control.

Conclusion/Discussion:

The method was robust and reliable for the quantitation of tramadol, O-desmethyl and N-desmethyl tramadol in breast milk. Of the 19 authentic breast milk samples analyzed, one was positive for tramadol and both metabolites.

High Resolution LC-QTOF MS Method for Quantitation of Benzodiazepine and Z-Drug Hypnotics

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Background/Introduction:

Benzodiazepines and Z-drug hypnotics are psychoactive drugs that are used for a wide range of clinical therapies including anticonvulsant, sedative, hypnotic, anxiolytic and anti-insomnia treatments. Due to their highly addictive nature, they are frequently misused or deliberately abused. Consequently, they are classified as controlled substances by regulatory and law enforcement bodies, thus necessitating the requirement for their rapid detection and quantification in the forensic context.

Objectives:

Traditionally due to their high specificity, sensitivity and wide scope of application, liquid chromatography - tandem mass spectrometry (LC-MS/MS) systems using multiple reaction monitoring (MRM) have generally been used for this purpose. The goal of this study is to evaluate the suitability of high resolution, accurate mass Quadrupole Time of Flight (QTOF) technology as an additional approach for the simultaneous detection, confirmation and quantification of benzodiazepine and non-benzodiazepine hypnotics in human serum.

Methods:

Spiked human serum (500 µL) was mixed with 1.5 mL extract solution (5% propan-2-ol in chloroform) and incubated for 15 min at 20 °C. The samples were centrifuged (10 min, 13,000 g) and the lower layer transferred to a glass vial with 50 µL 0.2 M hydrochloric acid. The sample was evaporated to dryness at 40 °C and the residue reconstituted in 500 µL methanol/water (1:9). The calibrators were treated in the same manner as the samples. Deuterated analytes were used as internal standards. MS-system: Bruker impact II UHR-QTOF, MS full scan mode 30-1000 m/z

Results:

Data processing was done with Bruker TASQ software. Identification was based on mass accuracy (3 mDa), retention time and diagnostic ions with ion ratios (tolerance: ± 30%). All 13 analytes have been successfully separated and identified, including the co-eluting compounds. Quantitation results showed very good z-scores<1 (except Clonazepam) and R2-values greater than 0.99.

Conclusion/Discussion:

The application of LC-QTOF full scan, accurate mass analysis has been shown to deliver accurate quantitative results for the analysis of 13 benzodiazepines and z-drug hypnotics in serum. The adoption of this quantitative high resolution workflow delivers additional analytical flexibility, as it also allows the possibility of retrospective quantitative/qualitative investigations to be undertaken without the requirement for further sample analysis.

High-Sensitivity Detection of Forensic Drug Panel in Human Whole Blood

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Background/Introduction:

The ability to achieve low levels of detection while accurately quantifying a wide concentration of drugs present in complex biological matrices is critical for any forensic laboratory. Recent improvements in instrument sensitivity have led to significantly improved detection limits for demanding quantitative workflows such as the detection of drugs of abuse in such samples.

Objectives:

The objective of this study is to develop a highly selective and sensitive LC-MS/MS workflow for the analysis of a comprehensive forensic panel of 49 compounds in human whole blood. The efficient sample preparation method in combination with the modified entrance optics and improved ion transfer from the ionization source on the modified hybrid triple quadrupole/linear ion trap system is shown to enhance limits of detection for low level analytes extracted from human whole blood.

Methods:

Control human whole blood samples were spiked at various concentration levels with a stock standard solution mixture and extracted by using a protein precipitation procedure followed by centrifugation; supernatant was evaporated and reconstituted in mobile phase for analysis. Analytes were chromatographically separated at 30˚C using a Phenomenex Kinetex phenyl-hexyl (50 x 4.6 mm, 2.6 µm) column. Mobile phases were ammonium formate in water and methanol with appropriate additives, 1 mL/min flow rate. Mass spectrometric detection was performed on a modified hybrid triple quadrupole/linear ion trap system operating in both positive and negative modes using polarity switching with multiple reaction monitoring (MRM) MS/MS method.

Results:

Following sample extraction, 5 µL of the reconstituted solutions were injected into the modified hybrid triple quadrupole/linear ion trap system. The MRM acquisition method enabled monitoring of two transitions per compound while the fast polarity switching capabilities of the instrument enabled combining both positive and negative polarity electrospray ionization modes into one comprehensive quantitation method. Calibration curves were generated for each of the 49 compounds to determine limits of quantitation (LOQ). The results demonstrate excellent linearity of the generated regression curves covering linear dynamic range of up to 5 orders of magnitude. Coefficient of variations (CVs) were found to be within 20% and accuracy ±20%, and R2 values greater than 0.98 for each of the 49 drugs targeted in this study. The combination of the efficient sample preparation and the modified hybrid triple quadrupole/linear ion trap system enabled to obtain sub ng/mL lower limits of quantification (LLOQ) for the vast majority of the compounds in the panel without compromising data quality.

Conclusion/Discussion:

The described method provides a robust quantitative platform with exceptional performance in speed, linear dynamic range and sensitivity for the accurate quantification of a panel of 49 drugs in human whole blood. The optimized method is shown to maximize the linear dynamic range capabilities of the instrument while retaining the required levels of accuracy and performance. The MRM acquisition method and the fast polarity switching capability of the modified hybrid triple quadrupole/linear ion trap system lead to improved sensitivity and quantitation limits.

Identification of Apomorphine and Nuciferine in Herbal an E-liquid, Extracted Resin and Electronic Cigarette Aerosols

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Background/Introduction:

Apomorphine a non-selective dopamine agonist that stimulates the dopamine receptors and improves motor function that primarily used to treat Parkinson's disease and nuciferine associated with dopamine receptor blockade are both found in the Blue Lotus Flower (Nymphea Caerulea). The Blue Lotus Flower is an Egyptian water-lily, whose flower has been used as a "natural" sleep aid and anxiety reliever. A wide array of commercial products such as capsules, powders, extracts and electronic cigarettes liquids (e-liquids) that contain Blue Lotus Flower extracts are available.

Objectives:

Presented is the identification and quantificaion of apomorphine and nuciferine the major psychoactive components of the Blue Lotus flower in an herbal e-liquids, extracted resin and electronic cigarette aerosols.

Methods:

An e-liquid and extract resin were purchased and aerosols were generated using a box mod e-cigarette. Direct Analysis in Real Time AccuTOFTM Mass Spectrometry (DART-MS) was used to identify the psychoactive components in the e-liquid and the resin. The extracted resin was dissolved in a 70:30 propylene glycol: glycerin mixture (10 mg/mL). Aerosol samples (n=3) were generated using a box mod type of e-cigarette at 90W and 203°C coil temperature. Aerosols were entrapped on a Cambridge filter (44mm). The method aerosol generating method was validated using CORESTA guidelines (N°81). The filter was extracted in methanol and the apomorphine and nuciferine were analyzed using a Waters AcQuity Xevo TQD system UPLC–MS/MS (Milford, MA).

Results:

No apomorphine was detected in the e-liquid and only trace amounts were identified in the resin. Nuciferine was quantitated at 63 ng/g in the e-liquid and 340 ng/g in the resin made e-liquid mixture. The generated aerosols contained 53±14 ng/g nuciferine for the e-liquid and 239±62 ng/g nuciferine for the resin made e-liquid mixture.

Conclusion/Discussion:

With the advent of e-cigarette devices capable of "vaping" e-liquids of natural products and extracted resins, their potential use for psychoactive drugs delivery may be realized. Funding: This work was supported in part by the National Institutes of Health [NIDA-Central Virginia Center on Drug Abuse Research: [P30DA033934] and the National Institute of Justice [2016-DN-BX0150]. 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.

Ion Suppression and Enhancement Study for 372 Synthetic Cannabinoids in Urine using Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF/MS)

Authors and Affiliations: Amanda Moore*, Michael Frontz, Janine DeKing, Greg Jellick, and Jeff Walterscheid, Ph.D.; Quality Forensic Toxicology, San Antonio, Texas

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, and paranoia. Due to the proliferation of these new drugs, the number of synthetic cannabinoids in forensic casework samples are suspected to be underreported and present a significant challenge to forensic toxicology laboratories. The ability to identify these synthetic cannabinoids in various matrices is essential in toxicology; and it is critical to evaluate ion suppression and enhancement contributions from the matrix to ascertain how it plays a role in the identification of these synthetic cannabinoids. The following is a systematic study in accordance with ANSI/ASB Standards 036 evaluating ion suppression and enhancement for 372 synthetic cannabinoids and their metabolites in urine using the LC-QTOF/MS.

Objectives:

To evaluate the ion suppression and enhancement of synthetic cannabinoids in urine matrices to determine the impact matrix has on reporting positive results.

Methods:

Using the novel Synthetic Cannabinoids Screening Library plates from Cayman Chemical (Ann Arbor, MI), a validated method and Personal Compound Database and Library (PCDL) complete with retention times for 372 synthetic cannabinoids was created. Two concentrations (~6 ng/mL and ~100 ng/mL) of neat samples were injected 6 times. Ten different urine matrices were subjected to an enzyme hydrolysis followed by an ethyl acetate/hexane liquid-liquid extraction in duplicate. Samples were then reconstituted with one of the two concentrations. Samples were 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. Ion suppression and enhancement was determined by evaluating the compound peak areas of the neat standards compared to the matrix samples reconstituted with the neat standards after sample extraction.

Results:

This study reveals the wide variety of ion suppression and enhancement that can be seen in synthetic cannabinoids with a range of ion suppression/enhancement from -75.65% to 65.68% at 6 ng/mL concentration and -79.20% to 66.25% at 100 ng/mL concentration. Though a wide range is displayed, out of the 372 synthetic cannabinoids involved 307 synthetic cannabinoids' percent ion suppression/enhancement were within 20% for the 6ng/mL concentration and 329 synthetic cannabinoids were within 20% for the 100ng/mL concentration. The majority of synthetic cannabinoids that share the lowest percent suppression/enhancement was consistent with the naphthoylindoles and benzoylindoles groups which contain many of the JWH compounds including JWH-018, JWH-073, and JWH-167. In contrast, the indazole-3-carboxamides compounds which include compounds such as AB-FUBINACA and MAB-CHMINACA had the greatest difference in percent ion suppression/enhancement at the 6 ng/mL concentration. Interestingly, these compounds decreased in percent ion enhancement and suppression at the 100ng/mL concentration. Figure 1 details a select group of synthetic cannabinoids frequently seen in forensic cases now. Metabolites like 5-fluoro ADB metabolite 7 and MDMB-FUBINACA metabolite 3 of indazole-3-carboxamides are suppressed more than other metabolites like 5-fluoro ADB metabolite 2 and MDMB-FUBINACA metabolite M1. The difference in ion suppression between metabolites may determine what metabolite should be included in an assay and explain why certain metabolites are non-detected in a positive sample. In our casework, 5-fluoro ADB metabolite 2 is more abundant than 5-fluoro ADB metabolite 7 therefore has been a more reliable metabolite for detection.

Conclusion/Discussion:

This study evaluated the ion suppression and enhancement for 372 synthetic cannabinoids in urine. Eighty-two percent of these synthetic cannabinoids were within 20% ion suppression and enhancement at 6 ng/mL concentration. The other 18% of synthetic cannabinoids vastly differed from 65.68% enhancement to -75.65% suppression. With the most variability occurring with the indazole-3-carboxamides synthetic cannabinoid group and the least variability occurring with the naphthoylindoles and benzoylindoles groups. Synthetic cannabinoids are of extreme forensic significance and the matrix in which these compounds are identified can greatly impact a positive result. More research is needed in this area. Further investigation by the laboratory will include the performing an ion enhancement/suppression study with the same synthetic cannabinoids tested with blood matrices and increasing our database to include upcoming synthetic cannabinoids such as MDMB-4en-PINACA.

Method Development and Validation for Simultaneous Confirmation of 15 Commonly Encountered Opioids by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

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Background/Introduction:

The opioid epidemic with which this country has struggled since the 1990s has been described as a three-wave epidemic by the Centers for Disease Control, beginning with prescription opioids, moving on to heroin just a decade ago, and evolving to fentanyl and its illicit analogs over the recent years. While the latest focus of forensic laboratories has been on identifying fentanyl analogs, it is vital not to minimize the importance that traditional opioids still play in the current opioid epidemic.

Objectives:

The objective of this study was to develop and validate a method for the confirmation/quantitation of 15 common opioids and metabolites using LC-MS/MS. Traditionally, it is necessary to derivatize opioids with an agent such as BSTFA for GC-MS detection. LC-MS/MS not only eliminates the need for derivitizing agents, but it also reduces the analytical run time and lowers the limit of detection/quantitation at least tenfold.

Methods:

Solid phase extraction was utilized on blood samples (1.0 mL) using 200 mg UCT Clean Screen® ZSDAU020 extraction columns. SPE columns were conditioned and washed before elution with a mixture of ethyl acetate, isopropanol and ammonium hydroxide. After evaporation to dryness, samples were reconstituted in deionized water for analysis using an Agilent 1260 Infinity II system coupled with an Agilent Ultivo LC-TQ. Chromatographic separation was achieved over 8.1 minutes using an Agilent Poroshell 120 EC-C18 (2.7µm, 3.0 x 50mm) analytical LC column heated at 30°C with a flow rate of 0.8 mL/min. A gradient elution was employed using 0.1% formic acid (FA) in water and 0.1% FA in methanol. Method validation guidelines were established based on the recommendations from the Scientific Working Group of Forensic Toxicology (SWGTOX). Parameters included a calibration model, bias, within run and between run precision, limit of detection (LOD), lower limit of quantitation (LLOQ), carryover, dilution integrity, interferences, ionization suppression/enhancement and processed sample stability.

Results:

All validation parameters were acceptable based on SWGTOX guidelines. Fentanyl, norfentanyl, buprenorphine, norbuprenorphine, 6-MAM, hydromorphone and oxymorphone were validated at a calibration range of 1.0-50 ng/mL; morphine, codeine, hydrocodone, dihydrocodeine, oxycodone, methadone, tramadol, and O-DT were validated at a range of 10-500 ng/mL. LOD/LLOQ was set at 1.0 and 10 ng/mL for the low and high curve, respectively. Bias was calculated to be <9% for all opioids. Within run and between run precision was <6% for all opioids except buprenorphine and norbuprenorphine, which were <15%. No significant effects were observed on accuracy and precision up to a dilution factor of 1:10. Interferences from biological matrices or from commonly encountered drugs of abuse were not observed; additionally, of 12 common fentanyl analogs investigated, only β-hydroxythiofentanyl interfered with norfentanyl. No carryover was detected for all opioids up to 10x the upper limit of quantitation. Ion suppression/enhancement was <8% for all opioids.

Conclusion/Discussion:

The method presented here allows for the reliable detection and quantitation of traditional, prescription opioids to at least 2.5 ng/mL. This is largely attributable to the sensitivity of the LC-MS/MS system, but also factors in the optimization of the solid phase extraction. The extraction optimization focused on the recovery and process efficiency of buprenorphine and norbuprenorphine, both of which are typically difficult to extract from biological matrices due to their unique chemistries. In addition to lowering the LOD/LLOQ of all compounds, this method greatly improves turnaround time and eliminates the use of halogenated solvents as well as potentially harmful derivatizing agents.

National Laboratory Certification Program Performance Testing for Specimens Containing Carboxylic Acid Metabolites of delta 8 and delta 9 THC.

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Background/Introduction:

Delta-8-tetrahydrocannabinol (Δ8-THC) is a compound found in low abundance in cannabis plant material. It is formed as a degradation product of delta-9-tetrahydrocannabinol (Δ9-THC) in the cannabis plant or by Lewis acid catalyzed conversion of cannabidiol (CBD) or Δ9-THC. In recent years, interest in the production and sale of Δ8-THC products has grown, resulting in the proliferation of Δ8-THC vape liquids and edibles. As the use of Δ8-THC has increased, some drug testing laboratories have begun to see more

positive cannabinoid samples containing this compound or its metabolites. The National Laboratory Certification Program (NLCP) was interested in determining the effect of Δ8-THC metabolites on urine cannabinoids testing in laboratories engaged in the federally mandated workplace drug testing program. Of particular interest was determining how testing urine for the presence and quantification of 11-nor-9-carboxy-delta 9-tetrahydrocannabinol (Δ9-THCCOOH) would be affected by the presence of 11-nor-9-carboxy-delta 8-tetrahydrocannabinol (Δ8-THCCOOH). We prepared a special set of performance testing (PT) samples in urine containing mixtures of Δ9-THCCOOH and Δ8-THCCOOH to determine analytical results when the two compounds are combined. The PT set was tested at laboratories accredited by the Department of Health and Human Services (HHS) under the NLCP.

Objectives:

To determine the extent of chromatographic interference when Δ9-THCCOOH is analyzed in the presence of Δ8-THCCOOH at a group of accredited forensic urine drug testing laboratories.

Methods:

A set of 11 PT samples in urine was prepared containing combinations of Δ9-THCCOOH and Δ8-THCCOOH shown in Table 1. The samples were shipped to 23 laboratories accredited under the NLCP. Laboratories were instructed to test each sample by their current immunoassay and quantify Δ9-THCCOOH in each sample using their current confirmation procedure.

Results:

The 11 samples gave positive results by immunoassay at all 23 laboratories. A review of results for Sample 1 (Δ9-THCCOOH at 100 ng/mL) and Sample 11 (Δ8-THCCOOH at 100 ng/mL) showed that both samples gave very similar results by immunoassay, indicating a high degree of cross-reactivity for Δ8-THCCOOH in a variety of immunoassay kits. In confirmatory testing, 20 of the 23 laboratories were able to successfully detect and quantify Δ9-THCCOOH in the presence of Δ8-THCCOOH in all samples. Three laboratories reported varying difficulties with the PT set. One laboratory was not able to confirm Δ9-THCCOOH in any of the samples that also contained Δ8-THCCOOH, due to chromatographic interference. Another laboratory was unable to confirm Δ9-THCCOOH when the concentration of Δ8-THCCOOH reached 20 ng/mL and Δ9-THCCOOH decreased to 80 ng/mL (Samples 3-10), due to chromatographic interference. The third laboratory was unable to confirm Δ9-THCCOOH when the concentration of Δ8-THCCOOH reached 70 ng/mL and Δ9-THCCOOH decreased to 30 ng/mL (Samples 8-10), due to ion ratio failures and chromatographic interference with the qualifier ions.

Conclusion/Discussion:

The presence of Δ8-THCCOOH can result in false negative results for Δ9-THCCOOH due to chromatographic interference and ion ratio failures. Although this was not experienced by any of the HHS-certified laboratories, previous reports in the literature indicate that laboratories using fast chromatography LC-MS/MS procedures may be at risk of false positives for Δ9-THCCOOH. Laboratories are encouraged to validate their cannabinoids confirmatory procedures to characterize and resolve interference caused by the presence of Δ8-THC and its metabolites.

Table 1. Concentrations of Δ9-THCCOOH and Δ8-THCCOOH in the Special PT Set

Sample Number	Δ9-THCCOOH (ng/mL)	Δ8-THCCOOH (ng/mL)
1	100	0
2	90	10
3	80	20
4	70	30
5	60	40
6	50	50
7	40	60
8	30	70
9	20	80
10	10	90
11	0	100

New Second Generation Specimen Validity Testing for Urine Drugs-of-Abuse Testing

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Background/Introduction:

During the past decade, the percentage of positive urine drugs-of-abuse (DOA) tests has remained stagnant while the overdose death rate and the number of opioid doses have steadily increased. The subversion of urine DOA screening is a major problem. Due to ineffective pre-analytical subversion detection, the first generation of specimen validity tests (SVTs) can only detect 2 of 18 classes of subversion.

Objectives:

A principal objective of the VDx GEN2-SVT™ panel is to increase the effectiveness of pre-analytical SVT testing to include 18 classes of subversion. Secondarily, the detection of subversion should be viewed as presumptive evidence of substance use disorder (SUD). These objectives can be achieved without undue burden on laboratories by adding only 2 new tests to the current panel of SVTs commonly used on automated instruments.

Methods:

The Validity Diagnostics (VDx) urine sample validity test (SVT) reagents are a second- generation urine SVT test panel. This unique set of reagents (VDx GEN2-SVTTM Panel) was developed to fill the gaps in the first-generation SVT reagents. Because the science of the VDx reagents is different from the first-generation reagents, the proficiency surveys from the College of American Pathologists (CAP) and the American Proficiency Institute (API) have placed the VDx reagents in their own category of reagents. The VDx improvements to the first-generation SVT reagents can be summarized as follows: pH - this reagent was improved by using two pH indicators instead of one indicator. The dual indicator reagent allows for very accurate readings at the low end (pH 3) and at the high end (pH 10.5). The readings in the middle (pH 5 – 7) are generally lower than the single indicator reagent. The VDx reagent can detect strongly buffered solutions. Urine CREATININE – this reagent was improved by add a decolorizing reagent to minimize interferences from hemoglobin. In addition, the linearity of the test was expanded past 250. Most importantly, it correlates with the SGI test below to detect creatine/protein loading of dilute urine samples. Specific Gravity Index (SGI) – this reagent is totally different from the classical specific gravity measurement. It uses an enzyme (beta-galactosidase) to measure total urinary sodium (Na+) and potassium (K+) ions. If this enzyme is affected by adulterants, the adulterants may also affect the enzyme used in the enzyme immunoassay drugs of abuse (DOA) tests. The SGI test can also detect dilution and salting. Oxidant History (OXH) – this reagent measures (in uric acid equivalents) the effect of an oxidizing reagent by measuring the decrease in urinary levels of markers (e.g., uric acid, ascorbic acid, glutathione, cystine, cysteine, and phenolics). This effect can be measured for up to 30 days. Otherwise, using the first-generation SVT reagents, the adulterant (nitrite equivalents) may not be detected after 12 hours. True Urine LD / SD (TRU-LD/SD) – these two tests in combination are used to detect three urinary tract proteins (UTP). The LD marker, UTP-IV, is from kidney tissue and has longer durability in urine. The SD markers (UTP-II & III) are short-life markers arising from the urinary tract which makes it difficult to manufacture, ship, and store synthetic urine containing the markers. The selection of UTPs was optimized to make the effectiveness of the VDx panel more robust in detecting synthetic or substituted urine. The sequence of testing for pre-analytical assessment of urine specimens for subversion would be: 1. Perform a VDx GEN2-SVT™ Urine Creatinine screen. This screen contains a decolorizing reagent which minimizes interferences from hemoglobin. This screen, in conjunction with #2 below, can be used to help differentiate dilution, in vivo creatine/protein water loading, salting or acid adulteration. 2. Perform a VDx GEN2-SVT™ Specific Gravity Index Screen. Interpretation of this result should be used in conjunction with #1 above. 3. Perform VDx GEN2-SVT™ True Urine LD, True Urine SD and Oxidant History screens. These screens help to differentiate dilution, oxidation, use of synthetic urine or substituted urine. 4. Perform VDx GEN2-SVT™ pH screen. This screening reagent contains dual indicator dyes that provide more accurate readings near pH 3.0 and pH 10.5.

Results:

The analysis of ~ 5,000 urine specimen results from several clinical laboratories using the VDx panel showed that ~ 10 - 20% of urine specimens had indicators of subversion and were considered “invalid” for subsequent urine DOA screens. This is in contrast to ~ 5% urine specimens showing subversion by traditional methods. Among the invalid urine specimens, many were determined to be substituted or synthetic urine by both the True Urine LD (associated with long-duration protein detection) and SD (associated with short-duration protein detection) tests. Some of these urine specimens screened positive for the prescribed drug class. However, LC/MS-MS analysis confirmed the presence of a very large level of the parent drug but no metabolites of the parent drug. The presence of a large level of parent drug without metabolites could indicate “pill-shaving” or “drug-spiking.” An example of “pill-shaving” involves a urine sample which screens presumptively positive for oxycodone but fails the True Urine LD and SD tests. The subsequent LC/MS-MS analysis of this urine sample yields a very high level of oxycodone but no oxymorphone or noroxycodone.

Conclusion/Discussion:

When used together, the VDx GEN2-SVT™ panel increases the effectiveness of pre-analytical SVTs to include 18 classes of subversion commonly used by individuals with a substance use disorder. The 18 classes of subversion defined by VDx are: 1: Adulteration with strong acid or alkali, 2: Simple dilution, 3: Masked dilution (e.g., creatine/protein-loading), 4: Adulteration with salt, 5: Adulteration with oxidant, 6: Adulteration with glutaraldehyde, 7: Adulteration with heavy metal, 8: Substitution with synthetic urine, 9: Substitution with the urine of another person, 10: Substitution with freeze-dried human urine, 11: Adulteration with cationic detergent, 12: Adulteration with protease, 13: Adulteration with acid buffer pH > 3.0, 14: Adulteration with alkaline buffer < pH 10.5, 15: Substitution using a catheter, 16: Presence of enzyme inhibitor, 17: Fraudulent prescription acquisition with DOA spiked synthetic urine, and, 18: Adulteration with glucuronidase inhibitor. The increased effectiveness can be achieved without undue burden on laboratories by adding only 2 new tests to the current panel of tests. Urine specimens passing the pre-analytical validity tests can undergo urine DOA screening with confidence. Urine specimens from donors failing subversion testing by the VDx GEN2-SVT™ panel should be considered for testing by LC/MS-MS to broaden the number of drugs detected.

One Stop Shot: DUID Confirmation and Quantitation LC-MS/MS Panel for Whole Blood, Urine and Oral Fluid

Authors and Affiliations: *Daniel Williams[1], Daniel Ayala[1], Jirair Gevorkyan[1], Kristen Burke[1] [1]California Department of Justice, Bureau of Forensic Services, Sacramento CA

Background/Introduction:

The California Department of Justice (DOJ) Division of Law Enforcement (DLE) provides toxicology testing for 45 counties through the Bureau of Forensic Services (BFS). The BFS has traditionally employed GC-MS methodology to provide consistent and quality results for human performance toxicology. However, in the last two decades, the BFS has seen a rise in DUID cases. In addition, the steady growth in polypharmacy, emergence of novel psychoactive substances (NPS), and proliferation of the opioid epidemic has stressed the toxicology systems that serve the State of California.

Objectives:

This increase in challenges and workload has prompted the BFS to adapt by employing state of the art liquid chromatography tandem mass spectroscopy (LC-MS/MS) technology to provide higher throughput service, with shorter turnaround, lower limits of detection and greater efficiency.

Methods:

Sample preparation consists of a simple acetonitrile crash extraction of 100 µL of blood, followed by centrifugation, dry down and reconstitution in starting LC conditions. Chromatographic and mass spectral resolution for targeted analysis are performed on a Waters Aqcuity ultra high pressure liquid chromatography (UPLC) system fitted with a Waters Acquity BEH C18 column and coupled to a Waters Xevo TQ-XS triple quadrupole mass spectrometer. The data processing and reporting logistics were designed to streamline quality assurance technical and administrative reviews, and the method was implemented into an ISO 17025 laboratory system.

Results:

The method was fully validated to American Standards Board (ASB) Standard 036 and international guidelines for validation of bio-analytical applications including bias, calibration model, carryover, interference studies, ion suppression and enhancement, limits of detection and quantitation, precision, dilution integrity and processed sample stability.

Conclusion/Discussion:

Through this approach, the BFS is able to confirm and quantify 56 analytes pertinent to DUID from the combined scope of 5 number of GC-MS methods previously employed by the BFS to serve as a “One Stop Shot” for DUID and DFSA toxicology testing in the State of California.

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Pharmacodynamics and Pharmacokinetics of 5F-MDMB-PICA in Male Rats

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Background/Introduction:

Synthetic cannabinoids (SCs) pose substantial risks to public health. Clinical information about the pharmacodynamics and pharmacokinetics of new SCs is lacking, which limits the ability to interpret findings from forensic cases. 5F-MDMB-PICA, first identified in biological samples during January 2018, persists in forensic casework as the most frequently encountered SC through the beginning of 2020.

Objectives:

Here we characterized the pharmacodynamics and pharmacokinetics of 5F-MDMB-PICA in rats that were fitted with jugular catheters. Rats received varying doses of 5F-MDMB-PICA, and blood samples were collected for analysis of plasma 5F-MDMB-PICA and its metabolites. Body temperature and catalepsy were evaluated as established indicators of cannabinoid-1 receptor (CB1) activation.

Methods:

Male Sprague-Dawley rats were fitted with jugular catheters and subcutaneous (s.c.) temperature transponders under ketamine/xylazine (75/5 mg/kg, i.p.) anesthesia. One week later, rats (n=6 per group) received single dose s.c. injections of 5F-MDMB-PICA (0.05, 0.1, and 0.2 mg/kg) or its vehicle, and blood samples were obtained at 15, 30, 60, 120, 240, and 480 min post-injection. The rats were double-housed under controlled temperature and humidity with 12 hour light dark cycles. Prior to each blood withdrawal, body temperature was measured using a hand-held reader, while catalepsy was scored based on immobility, flat body posture, and splayed limbs. In separate experiments, in vitro radioligand binding assays were used to determine CB1 affinity for 5F-MDMB-PICA and its metabolites. An analytical method was developed for 5F-MDMB-PICA and three metabolites: 5-OH-MDMB-PICA (METAB 2), 5F-MDMB-PICA Butanoic Acid (METAB 7) and 2-COOH-MDMB-PICA (METAB 8). The analysis was performed using a Waters Acquity UPLC® system coupled to a Waters Xevo TQ-S Micro Tandem Mass Spectrometer. The method was validated according to ASB validation standards. The calibration curve ranged from 0.5-500 ng/mL for 5F-MDMB-PICA and 1-500 ng/mL for all metabolites. The limit of detection was 0.1 ng/mL for all analytes. Plasma samples (200 µL) were acidified with 5% phosphoric acid and extracted into hexane/methyl tert-butyl ether/ethyl acetate (80:10:10). Samples were dried to completion, reconstituted in 50:50 0.1% formic acid in water/0.1% formic acid in methanol, and quantitated against the calibration curve.

Results:

5F-MDMB-PICA induced dose-related decreases in temperature that began within 15 min of injection and lasted up to 8 hours. Peak hypothermia occurred between 2-4 hours post-injection, and increasing drug dose increased the magnitude and duration of effects. 5F-MDMB-PICA induced dose-related increases in catalepsy scores. At the two highest doses, catalepsy scores peaked at 1 hour and lasted for up to 8 hours. Figure 1 demonstrates that 5F-MDMB-PICA has high affinity for CB1 receptors labeled with [3H]SR141716, whereas metabolites are at least 50-fold weaker. 5F-MDMB-PICA was detected in all plasma samples, and there was a linear increase in drug concentration as dose increased. Peak analyte concentrations were achieved at 2 hours post-injection for the 0.5 mg/kg (1.6 ng/mL) and 0.1 mg/kg (2.9 ng/mL) doses, and at 4 hour for the 0.2 mg/kg dose (5.1 ng/mL). 5F-MDMB-PICA butanoic acid was the only metabolite found at quantifiable levels, and was only detected after the highest dose of 5F-MDMB-PICA was administered.

Conclusion/Discussion:

5F-MDMB-PICA displays high affinity for CB1 receptors in vitro, and the drug induces dose-related hypothermia and catalepsy in vivo. Metabolites of 5F-MDMB-PICA are much weaker at CB1 receptors when compared to the parent compound. Of the metabolites tested in radioligand binding assays, 5-OH-MDMB-PICA showed the highest CB1 affinity, but this compound was not detected in any plasma samples. No metabolites were detected at concentrations above 2 ng/mL. Forensic toxicologists should consider these results when interpreting case findings for 5F-MDMB-PICA. More specifically, it will be important to examine the relationship between 5F-MDMB-PICA and its metabolites in forensic samples to determine whether there is differential abundance of these analytes in blood versus urine.

Potential presence of strychnine and brucine in homeopathic medications

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Background/Introduction:

Strychnos nux -vomica is native to Southeast Asia and India, but is commercially grown in China, Europe, the United States, and parts of Asia. Strychnine and brucine are the predominant alkaloids present, with the blossoms and seeds containing up to 1 – 1.5% strychnine, respectively, and brucine is predominantly present in the tree's bark. Strychnine's toxicity is displayed primarily as neuromuscular (muscle spasms or rigidity), but also as agitation or excitability, and may lead to respiratory failure and death. Strychnine is used at low doses therapeutically as a stimulant, laxative and for the treatment of various gastrointestinal issues; and has reported uses as a treatment for a host of other medical conditions. Doses as low as 15-20mg can cause severe clinical effects. A patient presented to the emergency department with severe vomiting, and was using "St. Ignatius bean" for treatment of constipation. No typical clinical neuromuscular findings expected from strychnine toxicity were observed, and the vomiting subsided within 8 hours. The St. Ignatius bean and other homeopathic "Nux Vomica" medications are available for purchase from herbal or homeopathy shops, as well as several grocery stores. St. Ignatius beans are typically used as a tea. The tea is prepared by directly steeping the bean in hot water or grinding to a powder and then steeped in alcohol before use. Nux Vomica preparations are typically in pill form, and ingested as a single dose (2-3pills) and up to an initial day's dose (15-16pills). As a note, in 1989, strychnine was banned by the US Food and Drug Administration (FDA) for use in nonprescription drug products.

Objectives:

To analyze various commercially available nux vomica products and St. Ignatius beans to determine the presence of strychnine and brucine using an UHPLC-MS/MS method.

Methods:

Commercially available formulations of Nux Vomica included: Boiron® Nux vomica 6c, 200ck, and ColdCalm®; and Hyland's® Nux vomica 30x. According to the Homeopathic Pharmacopoeia of the United States (HPUS), "x" and "c" denote the dilution of the original material, 1/10, 1/100, respectively, and the # denoted how many times the dilution was performed. The formulations were initially analyzed as the single labeled dose, then analyzed as the initial day's labeled dose. St. Ignatius beans (~3grams/bean) were analyzed following a 1 hour steep (either whole-bean in very hot water, or crushed bean in ethanol). Calibration curves were prepared from 0.01–100ng/mL. An alkaline liquid-liquid extraction was performed. Briefly, the dose was placed in 10mL of water and sonicated for 10min to aid in dissolution, then centrifuged at 3000g for 10min. To 1mL of supernatant, 50ng of oxycodone-d6, 0.2mL carbonate:bicarbonate buffer (3:2, pH 9.2), and 2.5mL ethyl acetate were added. The solution was rotator mixed for 10min, centrifuged for 5min, and the ethyl acetate was evaporated to dryness with nitrogen. The residue was reconstituted with 0.1mL mobile phase and 10mcL were injected for analysis. Analysis was performed in a Waters TQs-micro UPLC-MSMS system, using a Waters Acquity UPLC BEH C18 (1.7um, 2.1x50mm) column and a 0.1mM ammonium formate water(A):methanol(B) mobile phase. The gradient was 95:5 to 60:40 at 1.5min, then 0:100 at 3.0min, and held for 0.5min. The column flow rate was 0.5mL/min. The acquisition mode was multiple reaction monitoring, and positive mode electrospray ionization.

Results:

Strychnine and brucine were not detected in the single Nux Vomica dose, nor the daily dose analysis. Due to observed variability, the limits of detection were set at 0.1ng/mL. The strychnine concentration in the St. Ignatius steeped bean extracts were: hot water whole bean 550ng, and ethanol macerated 560ng. Brucine was not detected in either bean. Expected strychnine dose from these St. Ignatius beans would be <0.001mg.

Conclusion/Discussion:

The method was successful in the detection and quantitation of strychnine in the St. Ignatius beans, but not the nux vomica formulations. This may be due to the fact that, the bean contains up to 1.5% strychnine, and calculating the potential strychnine concentration forward, the maximal concentration in any one of these formulations would be 0.01ng/g. Brucine not being detected was also expected due to it being predominantly located in the tree bark.

Pre-Analytical Contamination Skews Forensic Interpretation of Barium in Biological Specimens

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Background/Introduction:

Barium is a soft alkaline earth metal commonly found as salts of barium carbonate, barium sulfate, and barium oxide. Barium salts have extensive industrial applications and are often used in the manufacturing of paints, ceramics, electronics, cement, and glass. Barium induces severe hypokalemia associated with muscle weakness and paralysis, severe ventricular dysrhythmias, hypotension, and respiratory failure. Barium exposures may be accidental, suicidal, or homicidal in nature and toxicological testing in biological specimens is required for confirmation. To properly test for barium without inadvertent contamination caused by collection devices, the recommended tube type for blood collection is a trace-metal free container such as the royal blue top tube. Since barium is used in glass manufacturing, the red and gray top glass tubes can potentially introduce barium contamination to the specimen. This poses a dilemma for medical examiners and coroners since red and gray top tubes, are frequently used for blood collection in death investigations. Contamination of barium from collection devices can result in elevated levels of barium in biological specimens, potentially resulting in the incorrect determination of the cause and manner of death in postmortem cases.

Objectives:

Ten years of forensic case data were reviewed to correlate the reported barium blood concentration with the associated blood collection device. Further, blood results were compared to the findings of other available matrices tested for barium to see if this secondary testing supported the elevated blood results.

Methods:

To evaluate the source of contamination, gray and red top test tubes were assessed for barium. Red and gray tubes were obtained and split into three sets of three. Groupings consisted of gray or red top test tubes containing blood that were placed either upright, upside down, or on the side. These were stored for five days and then analyzed by inductively coupled plasma mass spectrometry (ICP-MS).

Results:

Between 2010 and 2019, 183 forensic blood samples tested positive for barium by inductively coupled plasma mass spectrometry (ICP-MS) at values > 10 µg/L. Of the 183 positive samples, 98 were reported from gray top tubes/vials and 26 from red top tubes. Thus, 68% of positive blood samples were from collection devices that potentially introduced barium to the sample. Barium results ranged from 12 to 16000 µg/L in gray top tubes and 18 to 5600 µg/L in red top tubes. Of the 183 cases with barium blood results > 10 µg/L, only 28 were collected in trace-metal free royal blue top. Additional testing in alternative matrices is recommended when an elevated barium blood result is reported, especially if the blood is collected in an improper tube. Of the 98 cases using a gray top collection device, 26 had additional barium testing performed on either a liver, bile, urine, or hair sample. Of these cases, one case reported the ingestion of glass etching solution and had a gastric fluid result of >2500 µg/L, with a corresponding blood result of 570 µg/L. Additional testing in the other 25 cases did not confirm elevated blood results. Of the 26 cases collected in red top tubes, three had additional testing with only one confirming an elevated blood result. The barium contamination study showed results ranging from 319 to 916 µg/L for gray top tubes and 10 to 26 µg/L for red top tubes. Acid washed tubes containing blood in this study were negative for barium, thus confirming barium contamination from manufacturing of glass. Previous container screening studies have shown royal blue top tube, the recommended collection device for barium, having <2.5 µg/L.

Conclusion/Discussion:

Testing by ICP-MS is essential for identifying and quantifying elements in suspected poisoning cases. The type of container used for specimen collection in death investigations involving elements is just as important in these cases. The gray and red top tubes used for collection of specimens can skew barium results causing a misinterpretation in some cases. The recommendation for death investigations requires additional testing in appropriately collected alternate matrices if barium poisoning is suspected and pre-analytical contamination is a concern.

Prevalence of Four Designer Benzodiazepines in South Carolina from 2017-2019

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Background/Introduction:

Over the past 10 years, designer benzodiazepines (DBZD) have grown in popularity as a drug of abuse. Being unregulated with the possibility of increased potency, DBZD have the potential to be more dangerous and addictive. In 2017, etizolam and flubromazolam were the first DBZD to emerge in South Carolina. Over the following 2 years, these DBZD in addition to flualprazolam and clonazolam would grow in prevalence throughout the state of South Carolina.

Objectives:

The goal of this study was to evaluate the prevalence of DBZD in driving under the influence of drugs (DUID) and postmortem casework in South Carolina from 2017-2019. This three year time frame includes the emergence of these DBZD in South Carolina casework (2017-2018) until the period when they were added to an existing benzodiazepine confirmation panel (2019).

Methods:

The expanded benzodiazepine method consisted of 22 classical and DBDZ in blood and urine. Following solid phase extraction, samples were analyzed using an Agilent 1290 Infinity LC System coupled to an Agilent 6430 triple quadrupole mass spectrometer. Chromatographic separation was achieved using a flow rate of 0.4 mL/min on a UCT Selectra DA column (50 x 2.1 mm, 3 µm) with a gradient elution of 0.1% formic acid in water and methanol. Method validation guidelines were established based on the recommendations from the Scientific Working Group for Forensic Toxicology (SWGTOX). Data was compiled from 2017 to 2019 using postmortem and DUID reports to investigate the prevalence of DBZD in South Carolina.

Results:

During validation, the method described met all criteria outlined in SWGTOX guidelines. From 2017-2019, these DBZD were identified in 7 death cases and 111 DUID cases. Of the 111 DUID cases, 99 cases were reported in urine; the 12 DUID cases reported in blood were all from 2019. During 2017 and 2018, 28 DUID cases reported a DBZD; etizolam was reported in 75% of those cases. Over these two years, DBZD were reported in 14 of 46 counties in South Carolina. Demographics over this time period shows an average age of 23.4 years old (median age of 22), 75% involving a male, 78.6% Caucasian, 17.9% African American, and 3.6% Hispanic. In 2019, 83 DUID cases reported a DBZD; etizolam was reported in only 33% of the cases while flualprazolam was reported in 69% of cases. In 2019, DBZD were frequently reported with additional drugs, such as THC (n=63), opioids (n=34), classical benzodiazepines (n=31), stimulants (n=19) and additional DBZD (n=14). During this one year, DBZD was reported in 25 of 46 counties in South Carolina, with approximately 25% of the DUID cases found in York county. Demographics in 2019 shows an average age of 28.0 years old (median age of 25), 63% involving a male, and 75.9% Caucasian, 20.5% African American, 2.4% Hispanic and 1.2% Asian.

Conclusion/Discussion:

DBZD are developed with slight modifications that typically allow for antibody cross reactivity on common immunoassays, but will not confirm on assays directed toward traditional benzodiazepines. The existing benzodiazepine LC-MS/MS confirmation method was updated and validated in blood for the quantitation of clonazolam, etizolam and flubromazolam; flualprazolam was validated qualitative only. In urine, all four analytes were validated for qualitative purposes. Without the addition of these analytes to a specific confirmation method, unconfirmed benzodiazepine screens will continue to persist. After introducing the expanded method in Q2 of 2019, the number of confirmed cases to contain DBZD in the last 7 months of 2019 was more than triple that for the prior two years.

Prevalence of Ketamine in New York City by Retrospective Review of Postmortem (2003 – 2019) and DUID (2015-2019) Cases Submitted to the New York City Office of Chief Medical Examiner

Authors and Affiliations: Elba Arango, Zoila Rosario*, Allison Toriello, Gail Cooper Department of Forensic Toxicology, New York City Office of Chief Medical Examiner, New York City, NY 10016

Background/Introduction:

Ketamine is a dissociative anesthetic used in veterinary and human medicine since the 1970s. Its use has expanded to pre-hospital settings in emergency medical services and is now finding new clinical purpose as an analgesic alternative and antidepressant. As the country continues to combat mental health illnesses such as Opioid Addiction and Major Depressive Disorder (MDD), Ketamine brings hope to the mental health community to effectively manage chronic pain in the absence of opioids, and to decrease suicidal ideations in those suffering from MDD, respectively. However, its persistence as a recreational drug of abuse for its hallucinogenic properties remains. In the wake of expanding medicinal purposes for this recreational drug of abuse, the demographic diversity of New York City's population is also of particular interest in comparison to a previous study performed in 1997-1999. This retrospective study looks at the use of Ketamine by reviewing postmortem (PM) cases over a period of 17 years (2003-2019) and antemortem driving cases (driving under the influence of drugs; DUID) over a period of 5 years (2015-2019).

Objectives:

A greater understanding of the use of non-hospital administered Ketamine in NYC through a review of post-mortem and driving under the influence of drugs (DUID) cases.

Methods:

The NYC OCME provides forensic toxicology services for all five boroughs of the City of New York (population 8.6 million). In-house case management and laboratory information management systems were utilized to collate all cases where Ketamine was detected over a period of 17 years from 2003 to 2019 for postmortem cases and a period of 5 years from 2015 to 2019 for DUID cases. Demographic information recorded included sex, race, age, gender, borough, cause and manner of death, and other drugs detected. Analysis of Ketamine over the study period involved screening by gas chromatography-mass spectrometry (GCMS) with quantification by GC-NPD or GCMS with an LLOQ of 0.025mg/L.

Results:

Between January 1st 2003 and December 31st 2019, Ketamine was identified in a total of 142 deaths reported to the NYC OCME. Of these, 76% (N=108) account for cases where ketamine was not administered in a hospital or emergency medical service (EMS) setting. Of the 108 cases, 77% were male, with White, Asian and Hispanic accounting for 51%, 22% and 21% respectively. These deaths occurred mainly in the boroughs of Queens, Manhattan and Brooklyn at 31%, 29% and 25% respectively. A smaller data set for Ketamine positive DUID cases (N=35) was evaluated from January 1st 2015 to December 31st 2019. 80% (N=28) of these cases were male, with Asian, White and Hispanic accounting for 37%, 26% and 20% respectively. Table 1: Positive cases by year Postmortem CasesDUID Cases 200372012920152 200412013620162 200552014820179 2006120156201810 20076201611201912 2008320178 20093201814 2010420199 20117 Manner of death ruled an accident by intoxication was the leading cause of death over all postmortem cases (81%, N=87), followed by suicide (14%, N=15), and undetermined (6%, N=6). Of the latter, five had a history of drug abuse. The concentration ranges by case type are summarized in Table 2 below. Table 2: Blood concentration ranges (mg/L) where Ketamine was identified AccidentNaturalUndeterminedSuicide (not acute intoxication)Suicide (Acute intoxication)DUID <0.02 – 140.05<0.10 – 71<0.04 – 3.00.10 – 1.6 0.05 – 0.96 Examples of common drugs found in combination with Ketamine were: ethanol, methamphetamine, MDMA, cocaine, methadone, oxycodone, hydrocodone, PCP, 6-MAM, pentobarbital, fentanyl, verapamil, propofolol, and a number of benzodiazepines.

Conclusion/Discussion:

Overall, the retrospective study reveals that Ketamine's illicit use has not diminished in NYC. In the postmortem cases reviewed, 84% of positive cases were found amongst polysubstance drug users. Its presence in suicide cases accounted for 14%. A change in the racial demographics of positive cases is evident with an increase in use within the Asian and Hispanic population. Ketamine misuse remains overshadowed by misuse of more prevalent drugs in NYC.

Psychostimulant Abuse and Seizure Trends in Washington, D.C.: A National and Local Comparison

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Background/Introduction:

Psychostimulant abuse trends are growing in the United States. Increased production and distribution of drugs from Mexico are inundating multiple states and cities across the country. Available data from the Drug Enforcement Administration (DEA) and Centers for Disease Control (CDC) demonstrate the growing national trend in various cities. To determine if Washington D.C. was experiencing a rise in psychostimulant abuse, the toxicology laboratory at the Washington, DC Office of the Chief Medical Examiner conducted a data review. The review was inclusive of all postmortem (PM), driving under the influence (DUI), and drug-facilitated crime (DFC) cases positive for psychostimulants amphetamine, methamphetamine, MDMA, or eutylone between 2016 and 2019. Seizure data of these psychostimulants was collected by the Washington, DC Department of Forensic Sciences Forensic Chemistry Unit.

Objectives:

Compare national and local psychostimulant abuse trends; compare national and local psychostimulant seizure trends and; compare trends of psychostimulant abuse in D.C.'s PM, DUI, and DFC populations

Methods:

Statistical comparisons of national data sources (DEA and CDC) with local data sources (DFS Forensic Chemistry Unit data and OCME Toxicology Division data) using Microsoft Excel.

Results:

Data from Drug Enforcement Administration (DEA) field offices indicate increased methamphetamine seizures in the United States. In 2014, only three out of 23 national field offices reported more methamphetamine seizures compared to the previous year. In 2017, 14 out of 23 national field reported more methamphetamine seizures compared to the previous year. Local data collected from the Washington, DC Department of Forensic Sciences Forensic Chemistry Unit (FCU) indicates increased seizures of psychostimulants amphetamine, methamphetamine, MDMA, or eutylone in the region. In 2018, the FCU confirmed 32 psychostimulant exhibits. This number ballooned to 168 confirmed exhibits in 2019. From January 2018 to August 2020, the FCU confirmed 297 exhibits positive for one of these four psychostimulants. Of those positives, eutylone accounted for 48% and methamphetamine for 41%. National overdose data compiled by the Centers for Disease Control (CDC) shows consistent increases in overdoses involving psychostimulants. From January 2015 to January 2020, psychostimulant involved deaths increased from 4,402 reported to 16,528 reported – a 375% increase. However, the OCME toxicology laboratory found no significant trends in the PM and DUI cases. Over the four-year span, amphetamine, methamphetamine, MDMA, or Eutylone were detected in less than 2% and 2.5% of PM and DUI cases, respectively. In contrast, one or more of these psychostimulants were detected in over 11% of all DFC cases. Among the male subset of positive DFC cases, methamphetamine was the predominant psychostimulant detected; found in 90% of the cases.

Conclusion/Discussion:

Data from the DEA and the DFS FCU indicate increased seizures of psychostimulants. These increased seizure trends are corroborated by CDC overdose data, which show psychostimulant-involved deaths increasing year-over-year. However, the OCME toxicology division found no significant findings in Postmortem and DUI cases. However, these findings highlight an increased presence of psychostimulants in DFC cases within the District of Columbia. Though the local data is limited, methamphetamine trends exist within the male user population in Washington, D.C. Although further monitoring is needed, these results can be used to raise awareness of the rise in psychostimulant drug use in the DFC population.

Quantitative LCMSMS Validation of Flualprazolam and Detected Concentrations for All Case Types in Orange County, CA USA

Authors and Affiliations: *Fernando Manaloto, Orange County Crime Laboratory, Santa Ana, CA Dani Mata, Orange County Crime Laboratory, Santa Ana, CA

Background/Introduction:

Flualprazolam (IUPAC name: 8-chloro-6-(2-fluorophenyl)-1-methyl-4H-[1,2,4]triazolo[4,3- a][1,4]benzodiazepine; street name: Flualp) is characterized as a triazolo-benzodiazepine, similar to triazolam and alprazolam, but different in structure from other benzodiazepines such as diazepam. First synthesized in 1976, it is now considered as a 'novel' or 'designer' benzodiazepine. Flualprazolam is available for purchase through several Internet companies in the interest of research, though a search on online forums indicate that some individuals consume flualprazolam recreationally, describing the effects as being similar to being under the influence of alprazolam. Illicit manufacturing of flualprazolam is also documented, with the emergence of counterfeit pills being marketed as alprazolam or other drugs, but contain flualprazolam instead. There is little published information on the pharmacology, toxicology, or dependence potential for flualprazolam by itself, let alone in combination with other drugs. Validated quantitative methods are needed to assist with the interpretation of results.

Objectives:

Flualprazolam was first seen in Orange County in August of 2018 with 7 cases that year. In 2019, there were 123 cases in Orange County, and for the first three months of 2020, there were more than 70 cases. Due to this increase in cases, the laboratory decided to validate the addition of flualprazolam to the current benzodiazepine quantitation method, which can test antemortem and postmortem blood, urine, liver, gastric contents, synthetic blood, synthetic urine, and brain.

Methods:

The Toxicology section utilizes Liquid Chromatography-Quadrupole Time-Of-Flight (LC-QTOF) technology to comprehensively screen biological samples for over 300 drugs, including benzodiazepines and related compounds. Confirmation and quantitation of benzodiazepines and related compounds in biological samples are performed using DPX WAX tip extraction and analyzed on a Waters Acquity UPLC with a XeVo TQ-S MSMS in positive electrospray ionization mode. A Waters BEH C18 1.7 um, 2.1 x 100mm is held at 40 degrees Celsius with an organic mobile phase of acetonitrile with 0.1% formic acid and an aqueous mobile phase of water with 0.1% formic acid.

Results:

No matrix or other drug interferences (over 100 drugs tested) were observed during the validation. Flualprazolam has some of the same daughter ions as alpha-hydroxyalprazolam, but different retention times allow for distinct identification. No ion suppression or enhancement was observed during the validation as well. Recovery for all matrices was above 68%. Flualprazolam showed acceptable accuracy and precision in the established calibration range between the limit of quantitation of 4 ng/mL to a maximum calibration range of 256 ng/mL for all matrices tested. The calibration model verified was quadratic weighted 1/x2 with no forcing through zero. Once validated, all casework previously positive for flualprazolam was re-analyzed to obtain quantitative values.

Conclusion/Discussion:

Flualprazolam was fully validated using the ANSI guidelines for the addition of a drug into the existing method. Calibration integrity was observed for all matrices tested at the laboratory between 4 ng/mL and 256 ng/mL, with quality controls at 100 ng/mL and 10 ng/mL. Flualprazolam was added to the method for quantitation and confirmation, after a presumptively positive result from the LC-QTOF screen. The flualprazolam concentrations detected in death investigation and driving under the influence of drug cases will be presented on the poster.

Rapid Identification and Quantification of NPS in Human Whole Blood

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Background/Introduction:

Novel psychoactive substances (NPS) and other synthetic drug classes are a rising concern as thousands die from opioid overdose across the country. Some of these synthetic drugs have very high potency and thus only require a small amount for an accidental overdose. As the surge of novel synthetic opioids and other synthetic drug classes continue to pose serious public health and safety problems, timely and comprehensive drug screening approaches are critically needed in the forensic laboratory to quickly and accurately identify these emerging novel substances.

Objectives:

The objective of this study is to develop a comprehensive screening workflow using the TripleTOF 5600+ System for the detection, identification and quantification of NPS in human blood. The acquisition method generated comprehensive high-resolution MS/MS spectra that enabled accurate identification of the NPS through spectral library matching. The results demonstrate that this workflow is easily adaptable into a forensic toxicology laboratory and compatible with high throughput laboratory screening workflows.

Methods:

A stock solution containing the 30 NPS target analytes was prepared at various concentrations in methanol. The resulting solutions were subsequently diluted in control human whole blood, spiked with an internal standard stock solution and extracted from by using a liquid-liquid extraction procedure prior to analysis by LC-MS/MS. Analytes were chromatographically separated using a Phenomenex C18 (2.6µm, 3 x 50mm) column. Mass spectrometric detection was conducted on a TripleTOF 5600+ System operating in positive electrospray mode using SWATH acquisition. SWATH Acquisition enabled to collect MS and MS/MS information on every detectable peak within a sample with no loss in sensitivity, essentially creating a digital record of each sample analyzed. Samples were evaluated against four main confidence criteria: mass error, retention time, isotope ratio difference, and library score.

Results:

Control human whole blood samples were spiked with a mixture containing the 30 NPS and internal standards, extracted using a liquid-liquid extraction procedure and injected to build a data processing method. The liquid-liquid extraction procedure used in this experiment demonstrated excellent recoveries of the analytes used in this panel (>80%). Calibration curves were generated to evaluate the response, dynamic range and quantification performance (precision and accuracy) of the assay. The assay showed excellent precision (<15%) and accuracy (>95%), and R2 values greater than 0.99 for both the quantifier and qualifier ions for each of the 30 NPS targeted in this study. Using this comprehensive method, the 30 NPS used in this study were quantitatively identified and confirmation was achieved using MS/MS library searching with excellent library scores (>80% for all NPS analytes). The library fit scores (>80%) in addition to the passing confidence criteria provided excellent confidence for the definitive detection of the 30 NPS in human whole blood samples.

Conclusion/Discussion:

In this study, a comprehensive drug screening workflow using the TripleTOF 5600+ System for the analysis of NPS is described. The combination of a robust liquid-liquid extraction procedure with a comprehensive and high-quality MS/MS spectral acquisition strategy enabled sensitive and accurate detection of all target analytes in the NPS panel. Analyte extraction recoveries were demonstrated to be greater than 80%, allowing sub ng/mL detection limits of these drugs in a complex biological matrix while maintaining correlation and precision for all compounds across the calibration range. The results demonstrate the use of SWATH Acquisition in combination with SCIEX OS Software makes this workflow easily adaptable into a forensic toxicology laboratory and compatible with high throughput laboratory screening workflows.

Reducing evaporative crosstalk in urine assays using 96 well plates for LC-MS/MS analysis

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Background/Introduction:

LC-MS/MS analysis of volatile analytes is problematic when 96 well-plates are used. Solid phase extraction of urine with high drug concentrations can cause contamination of the elution solvent during evaporation as the compounds volatilize then redistribute in adjacent wells. This phenomenon, known as “evaporative crosstalk”, can generate false positive results. Controlling crosstalk in samples with very high concentrations is particularly difficult for analytes like sympathomimetic amines. Addition of HCl in methanol to extracted samples before evaporation is used to reduce crosstalk. The type of evaporator, conditions and solvents used can all contribute to crosstalk. The Biotage ACT Adapter (Biotage LLC, Charlotte, NC) was designed to reduce crosstalk during evaporation.

Objectives:

Evaluate the use of the ACT adapter and HCl in methanol to control evaporative crosstalk in urine samples with high concentrations of 8 drugs.

Methods:

All samples were prepared with drug free urine from healthy volunteers. Eight compounds were evaluated: amphetamine, methamphetamine, MDA, MDMA, MDEA, benzoylecgonine, morphine and hydromorphone. Calibrators and an extraction blank were aliquoted in the first column of a 96 well plate. Three samples spiked between 50,000 and 100,000 ng/mL were added in different areas of the plate. The rest of the plate was populated with drug free urine. Experiments were done with and without the Biotage ACT Plate Adapter using different concentrations of HCl in methanol. Briefly, 150 µL urine was treated with 165 µL of a master mix designed to be consistent with reagents added for enzyme hydrolysis: pH7 phosphate buffer, methanol (to mimic addition of internal standard solution) and water. Samples were not hydrolyzed. Samples were pretreated with 300 µL of 0.1% ammonium hydroxide. Next, 400 µL of each treated sample were loaded onto a 400 µL ISOLUTE SLE+ supported liquid extraction plate (Biotage) and processed using the standard SLE+ protocol with 2 x 600 µL of 90:10 dichloromethane:2-propanol for elution. This was evaporated using a Biotage SPEDry at 40°C upper, 60°C lower and gas flows of 40 upper and 30 lower. Reconstituted samples were analyzed with a Shimadzu Nexera UPLC and a Sciex 5500 triple quadrupole mass spectrometer (Sciex, Framingham, MA).

Results:

Initial experiments showed no evaporative crosstalk for benzoylecgonine, morphine or hydromorphone with or without the ACT Adapter and the addition of 10 µL of 1 mM HCl in methanol. Some crosstalk was detected in drug free urine samples adjacent to a spiked sample for MDA, MDEA and MDMA but this was reduced to <5 ng/mL with the ACT Plate Adapter. Concentrations in drug free wells between 2 and 200 ng/mL were observed for methamphetamine and amphetamine without the ACT Adapter. This was reduced to 1 to 100 ng/mL when the ACT Plate Adapter was used, but this was still too high for many clinical and forensic assays. Further work focused on reducing crosstalk for methamphetamine and amphetamine only. Next, the concentration of HCl in methanol was increased. Extractions were performed and either 10 µL of 0.25% HCl in methanol or 0.5% HCl in methanol were added to separate extracted plates prior to evaporation. Some crosstalk was still observed at both concentrations but was reduced to <30 ng/mL for both analytes when using 10 µL of 0.5% HCl in methanol and the ACT Plate Adapter.

Conclusion/Discussion:

The SAMHSA confirmation cutoff for methamphetamine and amphetamine in urine is 250 ng/mL. The ACT Plate Adapter reduced crosstalk for amphetamine, methamphetamine, MDA, MDMA and MDEA, but concentrations of amphetamine and methamphetamine were still too high for forensic analysis. Evaporative crosstalk was reduced to tolerable concentrations for amphetamine and methamphetamine (<30 ng/mL) using the ACT Plate adapter and 10 µL of 0.5% HCl in methanol.

Screening for 1100 Drugs in Urine using Standardized Chromatographic Separation and High-Resolution Accurate-Mass MS Analysis

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Background/Introduction:

The breadth and number of compounds that must be analyzed for forensic purposes is both huge and ever increasing. Maintaining the capacity to detect even a significant fraction of them can be both technologically and economically prohibitive. Many analytical systems can only detect a limited number of compounds in a given run, and laboratories can only afford to maintain limited stocks of authentic reference materials. Here we present an integrated method and library that can screen for over 1100 compounds of forensic interest in a single chromatographic run. The method uses a set chromatographic method with specific LC hardware to maintain retention times from system to system. Detection of analytes is by high-resolution accurate-mass mass spectrometry utilizing an Orbitrap-based mass spectrometer. Again, a specified acquisition method assures that fragmentation spectra are reproducible. Using the supplied compound database and library—developed on an identical system—compounds are identified and confirmed by a combination of retention time, accurate m/z, isotopic distribution, and spectral library and/or fragment ion matching.

Objectives:

The objective of this work was to develop a standardized liquid chromatography and mass spectrometry (LC-MS) method. To demonstrate the method's effectiveness, a partial method verification was performed using 50 representative drugs, in urine, selected to cover different drug classes, retention times, and polarities.

Methods:

The method specifies the LC and MS instrument hardware, software, and operating parameters. Specified chromatographic parameters include HPLC instrumentation, tubing lengths, mobile phases, column, and gradient. Mass spectrometry parameters include MS instrumentation, software, acquisition method, high-resolution spectral library, and compound database. The compound database includes the compound exact mass, chemical formula, retention time, and exact masses of main fragments for every compound. The data processing software screens for all compounds in the database and can also quantify compounds for which calibration curves are generated. The HPLC used is a Thermo Scientific Vanquish Flex Binary. Mobile phases were 2 mM ammonium formate with 0.1% formic acid in water and methanol:acetonitrile (1:1) for mobile phase A and B, respectively. The column was an Accucore phenyl hexyl 100x2.1 mm 2.6 µm run at a flow rate of 0.5 mL/min from 1 to 99% B. Compounds were detected on a Thermo Scientific Q-Exactive Plus high-resolution accurate-mass mass spectrometer. An inclusion list was used to trigger MS2 fragmentation spectra at specified retention times. Limits of detection (LOD), limits of quantification (LOQ), and limits of identification (LOI) were determined for 50 compounds, including opiates, benzodiazepines, anti-depressants, amphetamines, synthetic cannabinoids, and other NPS, in spiked urine. Concentrations ranged from 0.1 to 1000 ng/mL. Urine was processed by addition of internal standards followed by simple dilution before injection. The LOD was defined as the lowest concentration for which a peak was observed at a mass accuracy of 5 ppm. The LOQ was defined as the lowest concentration for which quantitation bias was <20%. The LOI was defined as the lowest concentration for which a compound passed: m/z of the parent (<5 ppm), isotopic pattern match, fragment ion presence, and MS^2 spectral matching.

Results:

A method capable of screening 1100 compounds in a single chromatographic run was implemented on a high-resolution accurate-mass LC/MS system. Run on a separate, identical system, the method was used to analyze 50 representative analytes of forensic toxicological interest, in urine. A partial method verification was performed. LODs ranged from 0.5 to 100 ng/mL. LOIs ranged from 5 to 1000 ng/mL.

Conclusion/Discussion:

Carefully duplicating instrumentation and operating conditions across multiple laboratories and systems allows a common method, spectral library, and compound database to be applied to analysis of compounds of forensic toxicological interest. This common bundle facilitates screening of over 1100 compounds in a single run and reduces the need for laboratories to purchase seldom-used reference materials until a compound's absolute confirmation is required. The method can also provide quantitative analysis of any compounds for which quantitation curves have been established.

Sensitive analysis of therapeutic and abused drugs in whole blood using Tip-on-Tip technology with LC/MS/MS

Authors and Affiliations: William E. Brewer*1, Evan DiVirgilio1, Kaylee R. Mastrianni1, and Casey Snodgrass2 1 DPX Technologies, LLC, Columbia, SC 2 Hamilton Company, Reno, NV

Background/Introduction:

LC/MS systems have made a significant impact on sample preparation requirements in forensic toxicology. In particular, highly sensitive LC/MS triple quadrupole instruments allow for low volumes of sample solutions, even when trying to achieve very low detection limits. In addition, the ability of HPLC separations minimizes the needs for rigorous extraction processes to purify samples for analysis. In this presentation, we demonstrate an improved automated protein precipitation method1 that provides rapid and sensitive analyses of comprehensive drugs and metabolites in whole blood. The method uses an automated protein precipitation procedure with Tip-on-Tip filtration to provide robust sample preparation while minimizing opportunity for human error. By using a very sensitive LC/MS system, we show a quick, low cost sample preparation procedure for accurately and reproducibly quantitating drugs and metabolites in whole blood.

Objectives:

To develop a rapid and automated extraction method for comprehensive drugs and metabolites in whole blood.

Methods:

Blank whole blood samples (from Utak) were spiked at various concentrations (0.5 ng/mL to 64 ng/mL) of 36 common drugs of abuse and their metabolites, including opioids and benzodiazepines (spiked using a mix of single standards ordered from Cerilliant). Using just 0.10 mL of whole blood, the samples were added directly to vials, and 10 uL of an internal standard mixture was added (at a concentration of 100 ng/mL containing morphine-d3, 6-mam-d6, oxycodone-d6, norfentanyl-d5, benzoylecgonine-d8, 7-aminoclonazepam-d5, fentanyl-d5, buprenorphine-d4, and temazepam-d5). The samples were placed onto a Hamilton Heater Shaker (HHS) and the automated method was started. The system mixed the solutions for 5 min, then added 25 uL of 0.2 g/mL of ZnSO4 and shaken for 30 sec. Subsequently, 0.25 mL of acetonitrile was added and the sample solutions were shaken for 60 sec to precipitate proteins. After mixing, a wide bore tip aspirated 200 µL of the supernatant. Then the tip was moved and positioned into a 300 uL filtration tip, making an air tight seal, then the Tip-on-Tip was moved over a vial rack, and the solution was dispensed into the corresponding vials. The solutions were solvent evaporated using nitrogen and heat, and reconstituted using 100 uL of 15% methanol. All analyses were performed using a SCIEX 6500+ triple quadrupole MS system coupled to an Agilent 1260 LC system (5 µL injection) equipped with a Phenomenex Biphenyl column (Kinetex 2.6 um, 50 x 3.0 mm). All extractions were performed using a Hamilton Nimbus96 liquid handler.

Results:

The automated protein precipitation and Tip-on-Tip filtration was performed in less than 10 min, processing up to 24 samples simultaneously (96 if using a 96 well plate) using a custom vial rack that holds 24 samples. Recoveries, which were limited to the efficiency of the protein precipitation, were over 50% for all of the drugs and metabolites. At a concentration of 3 ng/mL, %CVs were less than 10% for almost all of the compounds (meprobamate and carisoprodol were 15% and 11%, respectively). Limits of detection and quantitation were found to be less than 0.5 ng/mL for most of the 36 compounds, suggesting that less sample volume could be utilized for routine analysis. All linear regression values were greater than 0.99 even though most of the compounds did not use matching deuterated internal standards. Except for benzodiazepines, matrix effects were less than 20% ion suppression. The use of ZnSO4 reduced matrix effects and improved recoveries for basic drugs, in particular.

Conclusion/Discussion:

This study demonstrates a rapid, efficient, and sensitive automated method for analyzing common drugs of abuse and their metabolites in whole blood. By using protein precipitation with filtration, costs for sample preparation are greatly reduced. In addition, the reproducibility of this method was very good even though only 9 deuterated internal standards were used, which also reduces costs. Lower detection limits could be achieved by injecting a larger volume (for example, 10-20 µL) if necessary. However, the LODs achieved in this sensitive method suggest it is feasible to actually reduce the sample volume for routine testing. Further validation studies will be performed through collaboration with a forensic toxicology laboratory.

Separation of Ephedrine and Pseudoephedrine Isomers Using SCIEX SelexION Differential Mobility Spectrometry (DMS)

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Background/Introduction:

Ephedrine and pseudoephedrine are major alkaloids extracted from ephedra. These two compounds are cis-trans-isomers, and thus have different pharmacological properties. Differentiation and quantification of these enantiomers by high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) is challenging due to their weak column retention and co-elution, as well as their identical mass spectra, parent ions and fragment ions. As a result, the ability to separate these enantiomers is critical for pharmacological, toxicological, and forensic studies, as well as production quality control.

Objectives:

The objective of this study is to use the SelexION Technology in combination with the MRMHR workflow method on the TripleTOF 5600 System to provide accurate quantitation of the two isomers in plasma and urine samples. The developed workflow enabled accurate quantification of the two enantiomers with good linearity over a wide range of concentration from urine and plasma samples.

Methods:

Plasma and urine samples were spiked with stock standard solution mixture of ephedrine and pseudoephedrine and extracted by using a precipitation procedure with acetonitrile prior to being reconstituted in mobile phase for LC-MS analysis. The enantiomers were chromatographically separated at 15°C using a Phenomenex C18 (2.6µm, 3.0 x 50mm) column. Mass spectrometric detection was conducted on a TripleTOF® 5600+ System equipped with a SelexION differential mobility device and operating in positive electrospray mode with high resolution multiple reaction monitoring (MRMHR) MS/MS method. Multiple modifiers in combination with various compensation voltages were tested using the SelexION differential mobility device to determine the best separation conditions for the two enantiomers.

Results:

The spiked control urine and plasma samples were successfully used to determine the best separation condition. The LC-MS experiments found the two enantiomers are best separated when isopropanol is used as the modifier. Further optimization was performed by injecting one isomer on column and monitoring both compensation voltages. The best signal was observed at a compensation voltage of -44V and -41.8V for ephedrine and pseudoephedrine, respectively. The linearity of the assay was investigated using the control urine and plasma samples at concentrations ranging from 0.1 to 1000 ng/mL. Both matrices show good linear correlation with R2 values greater than 0.995. The recovery was found to be 88.38% - 108.8% in plasma and urine. Excellent reproducibility was achieved with CV within 5% with N=6. LC-MS runs with the isomers in matrix (plasma and urine) were acquired by collecting TOF MS data, with and without DMS on. These results demonstrated that the SelexION technology reduced matrix interference and helped improving the S/N ratio which resulted in better sensitivity in this analysis method, even when only analysing the parent ion signal. Last, the concentration of ephedrine and pseudoephedrine was studied in both biological matrices. This metabolism study showed highest concentration in plasma and urine 2 hours after administration. The study also showed that the concentration level of both compounds was higher in plasma than in urine.

Conclusion/Discussion:

In this study, accurate and sensitive quantitation of ephedrine and pseudoephedrine in plasma and urine samples was achieved using the SelexION Technology in combination with the MRMHR workflow method on the TripleTOF 5600 System. Excellent linearity, recovery, repeatability and matrix effects were achieved using the optimized separation method. The metabolism study performed showed that both isomers were highest in matrix 2hrs after administration and higher concentrations were observed in plasma vs in urine samples. In summary, this analysis method could provide a solution fast and accurate quantitation of ephedrine and pseudoephedrine in plasma and urine samples in forensic lab for narcotics analysis.

Simultaneous Analysis of 19 Novel Synthetic Cannabinoids in Urine Using SPE and LC-MS-MS

Authors and Affiliations: Abderrahim Abdelkaoui*, Ritesh Pandya, Brian Kinsella and Michael Telepchak

Background/Introduction:

Newly identified synthetic cannabinoids pose a significant threat to public health and safety, as their implications in drug overdose and adverse events continue to rise in the United States and around the World. The diverse chemical structures of synthetic cannabinoids have a significantly high impact on their potency and side effects. The 19 synthetic cannabinoids included in this study were previously un-reported in forensic toxicology casework in the United States. Currently, there are a few published methods available for the analysis of these novel compounds. However, identifying and extracting these compounds from various biological matrices is becoming more critical for accurate forensic investigations and clinical diagnostics. This poster will outline a solid-phase extraction (SPE) and LC-MS/MS method to analyze 19 synthetic cannabinoids in urine.

Objectives:

To develop a robust method for the identification and quantification of 19 novel synthetic cannabinoids in urine using SPE and LC-MS/MS.

Methods:

Ten blank urine samples were fortified at either 2.5 or 10 ng/mL of a working stock solution containing 19 synthetic cannabinoids (AB-PINACA N-pentanoic acid metabolite, ADBICA N-pentanoic acid, ADB-PINACA N-pentanoic acid metabolite, AB-FUBINACA oxobutanoic acid , 5-Fluoro ADBICA, ADB-BICA, 4-cyano CUMYL-BUTINACA , ADB-FUBICA, 5-Fluoro MDMB-PICA ,PB-22 3-carboxyindole metabolite, MDMB-FUBICA , BB-22-carboxyindole metabolite, UR-144 (XLR11) N-pentanoic acid metabolite, AKB-48 N-pentanoic acid metabolite, MDMB-FUBICA, AB-CHMINACA 3-methyl butanoic acid , BB-22, MA-CHMINACA, MDMB-CHMINACA). To this was added an appropriate volume of internal standard working solution and 1 mL of pH 6 phosphate buffer (0.1 M). Each SPE cartridge, UCT's Styre Screen® HLD highly crosslinked polymeric phase, was conditioned with methanol and equilibrated with deionized water (D.I H2O). After the sample loading, each SPE cartridge was washed with 2 mL of D.I H2O followed by 2 mL of H2O:ACN (80:20, v/v) containing 1% formic acid. Following the wash protocol, the SPE cartridges were dried under positive pressure for 5 minutes, and then eluted with 3 mL of ethyl acetate. The final eluate was evaporated to dryness at 40°C prior to reconstitution in 1 mL of the mobile phase. Instrumental analysis was performed with a Shimadzu LCMS-8050 Triple Quadrupole Liquid Chromatograph Mass Spectrometer. Separation of all 19 analytes was carried out using a UCT Selectra® C18 UHPLC column in under 10 minutes. The total run time, including 4 minutes of re-equilibration, was 14 minutes at a 0.4 mL/min flow rate.

Results:

Extracted urine samples fortified at 2.5 and 10 ng/mL yielded recoveries ranging from 86% to 115% and 95% to 112% respectively. Corresponding RSD values were less than 5% at both concentration levels. The quality control concentrations were designated at 2.5 ng/mL and 10 ng/mL to ensure that these compounds can be detected at low biological levels.

Conclusion/Discussion:

This poster outlines a simple SPE procedure for the analysis of 19 synthetic cannabinoids in urine using UCT's Styre Screen® HLD highly crosslinked polymeric SPE cartridges. All 19 compounds were analyzed and quantified in under 10 minutes using LC-MS/MS. The use of a UCT's Selectra® C18 UHPLC column resulted in excellent peak shape for all the compounds in the method, including baseline separation of any isobaric compounds. Excellent accuracy and precision values were obtained at the low levels evaluated. Since these analytes possess enhanced potency at low concentrations, their detection and suitable recovery from biological fluids are of utmost toxicological importance. This method will be beneficial to any lab looking to implement testing of these novel synthetic cannabinoids.

The Structural Similarities and Differences between a Variety of Electronic Cigarette Pods and Devices Contributing to the Possibility of Manipulation by Users

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Background/Introduction:

The rising popularity of e-cigarettes among young adults has resulted in a rapid transformation of device design into a category called "pod mods". Generally, the pod mods are closed system devices, not intended for users to refill or manipulate the e-liquid and are vaped until no e-liquid remains and discarded. Depending on the construction material of the pods, users can disassemble them to manipulate the e-liquid either by adding a drug other than nicotine (DOTN) directly to the e-liquid contained in a device's pod or adding a homemade blend with a DOTN to a re-usable aftermarket pod. The limited volume, typically less than 1 mL, and sleek concealable designs make the pod mod a preferred device for inhaling DOTNs. Limited research comparing the structural components of the different brands and designs has created a vacuum in understanding their efficacy as drug delivery systems and the potential dangers from poor quality construction.

Objectives:

The objective of this study was to compare the mechanical construction of two disposable pod mod e-cigarettes and five pods to assess user accessibility to the e-liquid.

Methods:

Two disposable e-cigarettes (Puff Bar, Mr. Fog) and 5 e-cigarette pods (Pax Era reference, JUUL reference, JUUL, Vuse, Suorin) were dissected. All the samples, excluding the two reference pods purchased locally, were obtained from users post-use. Each sample was dissected to create an "exploded" view to understand the housing construction, air exchange, heating mechanism, and contact points. Pictures were taken after each step to document the dissection process.

Results:

The two disposable e-cigarettes (Puff Bar & Mr. Fog) had similar mechanical compositions: a mouth piece, filter pad, connecting plug, a fabric-like air pathway, a coil and wick within the air way held in place with a silica plug and connecting the wires to the battery. The Puff Bar had a visibly burned wick at the point of contact with the coil and observable thick residues in the primary filter pad. Mr. Fog had no burned components and little to no residue in its filter pads. The JUUL reference pod had 10 components, while the used JUUL pod contained 8. They both consisted of a mouthpiece, connectors, a metal air pathway, wick, coil, and electrical parts all intertwined within a plastic casing. The reference pod had additional pieces connecting the mouthpiece to the air pathway and a piece connecting the coil to the electrical parts. Neither contained filter pads. The Pax Era pod consisted of a mouthpiece, four filter pads, two connecting parts, plastic air pathway, coil, wick, and electrical parts all within a plastic casing and was the same as the reference pod. The Vuse pod contained a mouthpiece, two connecting parts, heating chamber, and electrical parts within the plastic casing. The Suorin pod was a fused piece containing a coil and wick.

Conclusion/Discussion:

Two disposable e-cigarettes could be manipulated with some difficulty as the structural components are tightly assembled with an intricate design. Both the JUUL reference pod and the used JUUL pod contained silicon components and were easily disassembled, making these pods easy to manipulate for e-liquid adulteration. The Pax Era and Vuse pods hard plastic components required breaking in order to access the interiors, resulting in them being less likely to be manipulated. The Suorin pod was nearly impossible to dissect; however, this was a refillable pod any e-liquid could be added. Despite efforts to improve and control quality and user manipulation, many pod mod models still enable users to add DOTNs. Opinions are the authors and not those of the NIJ. This project was funded by NIJ#2018-75-CX-0036 and NIH P30DA033934.

Ultra-Sensitive Analytical Methodology for the Quantification of 11-nor-9-carboxy-THC (THCCOOH) in Oral Fluid

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Background/Introduction:

Cannabis is the most commonly abused recreational drug worldwide. Detection of its use can be performed in several biological matrices such as blood, urine and oral fluid. While blood and urine are useful in determining cannabis consumption, oral fluid has gained considerable attention as a quicker and less invasive means of monitoring cannabis use. More specifically, oral fluid 11-nor-9-carboxy-THC (THCCOOH) has been proposed as the marker quantified for cannabis intake since it is not present in subjects passively exposed to cannabis.

Objectives:

The objective of this study is to develop a highly sensitive LC-MS/MS method for the quantification of THCCOOH in oral fluid. The method is shown to provide unique advantages in the ability to quantify pg/mL levels of THCCOOH without the need for derivatization.

Methods:

Blank oral fluid samples were spiked with THCCOOH at various concentration levels. 750 µL of Quantisal® buffer was added to 250 µL of each spiked oral fluid sample spiked with an internal standard solution. Solid phase extraction (SPE) was performed to extract the analytes from oral fluid and the resulting solutions were transferred into autosampler vials for analysis. Analytes were chromatographically separated at 60˚C using a Phenomenex Kinetex C18 (50 x 2.1 mm, 2.6 µm) column. Mobile phases were 0.01% acetic acid in water and 0.01% acetic acid in methanol, 0.5 mL/min flow rate. Mass spectrometric detection was performed on a modified hybrid triple quadrupole/linear ion trap system operating in negative mode with multiple reaction monitoring (MRM) MS/MS method.

Results:

Following the SPE procedure, 40 µL of the reconstituted solutions were injected into a modified hybrid triple quadrupole system. An MRM acquisition method was used to monitor levels of THCCOOH in the extracted samples using negative electrospray ionization mode. Calibration curves were generated for THCCOOH to determine the overall assay sensitivity and limits of quantitation (LOQ). The results demonstrated excellent linearity of the generated regression curves. Coefficient of variations (CVs) were found to be within 20% and accuracy ±15%. The combination of high-throughput sample preparation procedures and the improved sensitivity on the modified hybrid triple quadrupole/linear ion trap system enabled to obtain lower limits of quantification (LLOQ) below 10 pg/mL for THCCOOH in oral fluid.

Conclusion/Discussion:

The described method provides a comprehensive solution for oral fluid drug testing in routine workspace, pain management, drug treatment, and forensic testing laboratories that is suitable for high-throughput screening of THCCOOH. The method allows forensic toxicologists to accurately monitor chronic and acute cannabis exposure with a high level of sensitivity and specificity and provides a rapid a reliable means of differentiating passive environmental cannabis exposure from active cannabis consumption.

UPLC-MS/MS Method for Detection and Quantitation of 90 Drugs and Metabolites in Acidified Oral Fluid with TAC Technique for Matrix Normalization

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Background/Introduction:

Use of oral fluid for drug abuse and compliance monitoring has increased and recent United States authorization of oral fluid as an alternate matrix in employment testing is likely to further expand use of this matrix. A classical urine drug screening protocol, based on presumptive testing of drug classes by immunoassay, has also been adopted in oral fluid testing programs. However, our experience with conversion from presumptive to definitive (UPLC-MS/MS) drug screening for initial testing has demonstrated enhanced selectivity of detection and reduced incidence of false negatives.

Objectives:

To detect and quantify a panel of illicit and pharmaceutical drugs and metabolites (analytes) in oral fluid by UPLC-MS/MS analysis using automated sample preparation that applies a previously published TAC (Threshold Accurate Calibration) technique for normalization of matrix effect. The pre-analytical objective is stabilization of analytes during collection and storage prior to testing. The method is intended for a range of forensic and clinical applications with a software option for application-dependent analysis of selective analyte panels.

Methods:

Neat oral fluid (1 mL) is acidified to a pH below 4.0 by addition of formic acid (10%v/v). Analytes from multiple drug classes including: antidepressants, antipsychotics, benzodiazepines, sedatives, gabapentinoids, muscle relaxants, opiates/opioids, phenylethylamines, cocainics, etc., are analyzed by UPLC-MS/MS, using a Waters ACQUITY UPLC BEH Phenyl column (3.3 min gradient) interfaced with a Waters Xevo TQD mass spectrometer. The definitive method monitors MS/MS transition ions for selective identification and quantification of analytes including the diastereomer and isobaric analytes. TAC technique involves automated preparation in a 96-well plate format of dual aliquots (40 µL) of calibrators, controls and case specimens in adjacent neat and spiked wells. Following addition of reference analytes to the spiked wells, and an injection-recovery monitor (methapyrilene) to neat and spiked wells, samples were diluted (1:7) before UPLC-MS/MS analysis. Analyte-specific parameters include a lower limit of quantitation (LLQ) range of 2.5-250 ng/mL, a validated limit of detection at 40% of LLQ and a quantitative range of 100-1000 %LLQ. A software template was developed for analysis of exported MS/MS acquisition data to manage case review and quality control and the software allows selective analysis of the analytes requested for testing.

Results:

The assay was validated for linearity, accuracy, precision, sensitivity, specificity, and carryover according to SWGTOX guidelines. Chromatography is optimized for separation of isobaric analytes with retention times ranging from 0.8-2.5 min. In 25 analytical runs, calibration performance was verified with a R2 >0.98 and precision at 40 %LLQ (mean 39 %LLQ), 125 %LLQ (125 %LLQ), 500 %LLQ (531 %LLQ) and 1000 %LLQ (964 %LLQ) with all CVs <15%. Dilution accuracy and precision was determined at analyte concentrations of 1500 %LLQ and 2500 % LLQ with mean concentration of 1460 %LLQ and 2580 %LLQ (CVs, 9% and 7%, respectively). Absolute matrix effects in oral fluid from 8 donors ranged from -68% to 69% with significant within-analyte variability. However, normalization of matrix effect by application of the TAC technique was demonstrated with mean analytical recovery of 99% (SD 6.6%) and 102% (SD 7.7%) at analyte concentrations of 125 %LLQ and 375 %LLQ, respectively. Carryover following high calibrator analysis averaged 1.6%LLQ (range 0-19%LLQ). Three rounds of blinded proficiency testing, using acidified oral fluid pools fortified with varying analyte combinations, showed identification concordance of 100%, concentration determinations within 20% of target and absence of false positives. Pre-analytical stability of analytes (at 400 %LLQ) in neat and acidified oral fluid pools was evaluated over 14 days at 4°C. Neat oral fluid showed reduced analyte response (<80%) for 18 analytes and an increasing trend for 9 analytes. In acidified oral fluid, analyte response within 20 % of baseline was found for all analytes.

Conclusion/Discussion:

A definitive method for identification and quantification of 90 drugs and their metabolites in oral fluid has been developed and validated. The method employs a pre-analytical specimen acidification for stabilization of analytes and an automated method for matrix normalization without the use of analyte-match stable isotope standards. Rapid chromatographic separation and software-facilitated data management allow routine application of UPLC-MS/MS selectivity and sensitivity in oral fluid drug detection and quantitation.

Urine Variability Compromises β-Glucuronidase Performance Causing Inaccurate Drug Tests

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Background/Introduction:

β-glucuronidase is used to remove glucuronic acid from phase II metabolites present in biological fluids prior to mass spectrometry detection to increase signal sensitivity. The result accuracy depends on the enzyme hydrolysis efficiency, which varies among different enzyme sources. Recently, we have discovered that enzyme hydrolysis efficiency could be reduced in different patient urine samples due to high heterogeneity in terms of physical and chemical properties; such as pH, specific gravity and creatinine level. This reduced hydrolysis efficiency could lead to inaccurate quantitation of metabolites. We present data to show variability within human urine samples can impact enzyme hydrolysis efficiency on commercially available β-glucuronidases. The experiment compares hydrolysis efficiencies between three purified β-glucuronidases in the same set of patient samples.

Objectives:

Demonstrate that variability within patient urine samples can affect the hydrolysis efficiency of some β-glucuronidases, causing inaccuracy during urine drug confirmation analysis. The new β-glucuronidase demonstrates higher tolerance toward patient sample variability, improving hydrolysis efficiency despite biological sample variability for accurate results at room temperature.

Methods:

Two synthetic urines and a certified drug free urine were fortified as control samples. Patient samples with and without additional 500 ng/mL of each glucuronide standard; hydromorphone, codeine and oxymorphone (Cerilliant) were also tested. 50 µL of fortified urine was mixed with 5 µL of purified β-glucuronidase, 150 µL of optimal hydrolysis buffer, and 10 µL of internal standard in methanol. Internal standard comprised of all corresponding deuterated analytes of interest. Purified β-glucuronidases included IMCSzyme® RT, Brachyspira pilosicoli (BpGUS) and Patella vulgata (PvGUS). Samples were hydrolyzed for 15 minutes at room temperature. Hydrolyzed urine samples were extracted on Hamilton Microlab NIMBUS96 using WAX/RP INtip™ . Samples were eluted with 400 µL of 1% formic acid in acetonitrile. The elute was evaporated and reconstituted with 50 µL of methanol followed by diluting with 400 µL of mobile phase A (0.1% formic acid in water). 10 µL of sample was injected on a Thermo Scientific™ Vanquish™ UHPLC system coupled with a Thermo Scientific™ Endura™ Triple Quadrupole Mass Spectrometer using a Phenomenex Kinetex® 2.6 µm Phenyl-Hexyl 100 Å, 50 x 4.6 mm column. Mobile phase B was 0.1% formic acid in acetonitrile.

Results:

IMCSzyme® RT tolerated the variability within patient samples, achieving 100% conversion for hydromorphone, codeine and oxymorphone in all patient samples in less than 15 minutes at room temperature. The other β-glucuronidase exhibited inconsistent hydrolysis conversions depending on the patient sample and achieved hydrolysis conversions of 85% or less on hydromorphone, 65% or less on oxymorphone and 20% or less on codeine.

Conclusion/Discussion:

There are significant analyte recovery differences due to patient variability depending on the enzyme being used. False negatives for codeine were also reported due to lower hydrolysis efficiency in some urine samples. One out of three purified enzymes tested is tolerant to the variability that comes with biological samples and rapidly hydrolyzes all glucuronidated drugs. This improves accuracy, reduces hydrolysis time, eliminates incubation equipment and enables full automation for urine drug confirmation.

Using Web-based Modeling Software to Predict Retention Times of 70+ Inhalants of Abuse on 4 Unique GC Stationary Phases

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Background/Introduction:

Screening for volatile inhalants of abuse, as well as analyzing blood alcohol content, is commonly performed in forensic toxicology laboratories using headspace gas chromatography with flame ionization detection (HS-GC-FID). The analyses are generally performed using dual columns with specialized stationary phases that optimally separate these volatile compounds. While separation profiles of standard blood alcohol screening compounds are usually well characterized by column manufacturers on these application specific columns, elution profiles of inhalants may not be as readily available. In addition, providing example chromatograms with static run conditions may not suit laboratories, who want to experiment with faster run times, column dimensions, carrier gases, etc. These issues can be solved by using computer modeling software to predict retention times of compounds of interest on various stationary phases. In addition to the ability of the web-based software to help select a column and provide an optimized separation of compounds of interest on a specific stationary phase, the software can also be used to make changes to analytical conditions and observe the effect on elution, making it a valuable tool for method development and optimization.

Objectives:

The primary goal was to develop retention time models for inhalants of abuse and blood alcohol analytes of interest on four unique GC stationary phases, using web-based modeling software. Accuracy of the models was verified against actual analyses. Demonstrations of the utility of the modeling software for optimizing separations, speeding up methods, and translating to other carrier gases and column dimensions will be presented.

Methods:

To build a database for computer modeling of chromatographic separations, each of the following fused silica capillary columns was installed into an Agilent 7890A GC with a 5975C MSD: Rtx-BAC1, Rtx-BAC2, Rtx-BAC PLUS 1, Rtx-BAC Plus 2. More than 70 volatile inhalants of abuse, including solvents, refrigerants, nitrites (aka “poppers”) and their metabolites were analyzed on each column using three different temperature programmed run conditions. Two of the analyses were used to create a retention model based on thermodynamic indices of analytes and the third analysis was used to verify accuracy against the theoretical model. Once the models were finalized, Pro EZGC, a web-based modeler, was used to optimize separations on each column, decrease analysis times, translate to different column dimensions or carrier gases, and make user inputted adjustments to parameters such as carrier gas flow rate and oven ramp rates.

Results:

Confirmation runs were in excellent agreement with the theoretical modeled analysis, demonstrating acceptable accuracy of the retention time models. Selection of various compounds of interest in the software successfully generated optimized separations on each of the four columns, allowing the user to choose the column or column set that best fits their needs. Successful demonstration of method optimization using the web-based modeler is provided, including speeding up analyses, translating to a different carrier gas, and user manipulation of parameters.

Conclusion/Discussion:

Computer modeling of compound retention times in gas chromatography is a valuable tool to aid in column stationary phase selection and method development/optimization. Use of this software greatly reduces the time required for manual method development, as users can input changes to the method and see the results instantaneously. With libraries of 70+ volatile compounds of abuse on four columns, users can select or input compounds of interest and then calculate elution profiles on each column. The software will present the number of coelutions on each column, allowing the user to select the most appropriate column for their analysis. Libraries are also expandable and compounds can be added in the future if there is user demand. The modeling solutions are presented on Restek GC columns, but model prediction will work on any manufacturer’s GC unit, when using the recommended conditions.

Validation of a Polyethylene Glycol Method Used to Positively Identify Patients with their Urine Sample for Toxicology Testing

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Background/Introduction:

Current laboratory methods can identify attempts to dilute and tamper with urine samples with substances such as water, oxidants, soaps, etc. However, the substitution of a "clean" urine from another individual or synthetic urine is still an issue. Collection of urine samples can be supervised in cases where a patient may attempt to switch their samples, but this can be difficult and embarrassing, and does not completely ensure the patient has not used a concealed device to provide the sample. A newer approach to positively correlating patients with their urine samples is the use of a low molecular weight polyethylene glycol (PEG). The FDA lists PEG as an inactive ingredient, and is commonly used in everyday over-the-counter medications. PEG is not metabolized by the body, and is cleared quickly through the kidney. This makes it an ideal urine marker. A pill or liquid is ingested by the patient at least thirty minutes prior to providing a urine sample. Each pill/liquid contains a unique combination of PEGs at various molecular weights. Each is barcoded with which version(s) are included in the sample. This allows definitive tracing of the urine sample back to the providing patient.

Objectives:

In order to use PEGs to successfully identify and connect urine samples with their donor, a validated PEG method is required. The method is considered a lab-developed test.

Methods:

Instrument: Agilent 6420 Analytical Column: Agilent Eclipse Plus c18, 2.1 x 50 mm, 1.8 µm Mobile Phase A: 0.1% Formic Acid in WaterMobile Phase B: 100% Methanol Flow Rate: 0.5 mL/min Column Temperature: 30°C (+ 0.8C) ESI Source Parameters: Ionization mode: Positive ESI Capillary voltage: 3000 V Drying gas flow: 10 L/min Drying gas temperature: 300 °C Nebulizer gas: 40 psi Gradient: TimeA %B % 0.00955 3.10595 3.60955 Acquisition MRM and Retention Time: Image 1 Sample Preparation: 1.500 µL of urine sample and calibrators/QCs dissolve in urine is mixed in an Eppendorf tube with 20 µL of 100 µg/mL solution of TEGDME as ISTD & precipitated with 25 µL ZnSO4 solution (0.1M Aqueous). 2.Let the solution cool for 5 minutes at 2-8 °C. 3. Solution is thoroughly mixed and centrifuged for 5 min at 3,000 rpm. 4.Dilute 400 µL of supernatant with 400 µL of LC/MS grade H2O 5.Transfer to 96-well plate. Cap the 96-well plate and mix well. 6.Inject 1 µL

Results:

Each PEG was run individually in DFU to determine Interferences and ensure Selectivity and Sensitivity of each analyte. The method was found to be free of interfering substances and exact in its identification of each PEG molecular weight at their specified retention times for both the quantifier and qualifier ions. Matrix Effects and Ion Suppression/Enhancement were tested using a series of sample preparations to determine that the internal standard would compensate for any ionization of other effects caused by various patient sample matrices. Ten patient samples, including a DFU, were tested at three different concentrations, showing that matrix effects had a CV <5% (acceptable CV <20%). Additionally, the same three concentrations were tested in LCMS grade water to examine suppression/enhancement, and found to have a CV <2% (acceptable CV <20%). Linearity was determined by running six calibration curves to establish regression type, linearity, and reproducibility. R values were acceptable at 0.98000 or better. Curves were required to be linear, without inclusion of the origin. The method showed excellent results with a CV <10% (acceptable CV <20%). Precision and Accuracy was determined by testing a total of eighteen sets of quality controls in sets of three over three days. The method was shown to have excellent results with a CV <10% (acceptable CV <20%). Carryover studies were conducted using twice the concentration of the highest calibrator, and found to be negative. However, it should be noted that PEG is particularly viscous. Incorrect dilution of the analytes can create contamination issues, both in the lines of the liquid chromatography tower and of the detector in the mass spectrometer. Fortunately, the risk of carryover from patient samples is not possible as the concentrations of PEG are limited by the ingested pills/liquid. Stability was examined at room temperature (25°C) for 14 days, and found to be stable throughout.

Conclusion/Discussion:

The method was found to be simple, reliable, and fast. The PEG markers are reliable and stable in urine over time, and the method studies show that the markers are clean and easily identifiable. Use of PEG markers to accurately match patient samples with their donor could be a powerful tool in reducing instances of falsification.

SOFTEMBER PLATFORM ABSTRACTS SESSION 1

A Single Blood and Urine Method of All Recommended and Additional Impairing Drugs in DUID Casework

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Background/Introduction:

In spite of the illegality of driving under the influence of drugs (DUID), more than 12.6 million people in the United States did such in 2018. In order to deter such behavior, Drug Recognition Experts are employed to obtain biological samples from suspected drivers for forensic toxicological analyses of impairing substances. Federal organizations such as NSC-ADID and OSAC have put forth recommended scopes for DUID toxicology testing. However, historical methods used to detect these substances do not cover the entire scope within a single analysis. Many methods also required time and resource consuming extraction methods. With recent improvements in technology, a more inclusive and efficient method is now feasible.

Objectives:

The aim of this research is to develop a single, comprehensive multi-class, and rapid method that meets and exceeds the recommended scope and sensitivity of testing for DUID casework with the goal of increased efficiency and sustainability as drug-impaired driving and polydrug use increases.

Methods:

One hundred and fifty microliters of blood or urine were extracted using acetonitrile protein precipitation and subsequent in-vial filtration, followed by nitrogen dry-down and 20:80 organic:aqueous reconstitution. The LC-MS/MS system was a Sciex Nexera X2 LC-30 with a Sciex QTRAP 6500+ mass spectrometer utilizing an Ion DriveTM Turbo V electrospray ionization (ESI) source in both positive and negative modes. Chromatography utilized a gradient on a Kinetex Phenyl Hexyl 100 Å LC Column (100 x 2.1 mm, 2.6 µm) column. Data acquisition and processing with customized query for automation incorporating quality assurance was performed. The analytical method was fully validated to SWGTOX and international guidelines including: bias (accuracy) and precision, dilution integrity, carryover, interferences, selectivity, limit of detection, lower limit of quantitation, matrix effects, processed sample stability, and linearity experiments. To demonstrate applicability, the described method was applied to previous proficiency test samples and authentic case samples.

Results:

This analytical method incorporates 127 drugs and metabolites, resulting in a list of target analytes that exceed the recommended scope, while meeting OSAC and international validation requirements. The method was validated for both antemortem and postmortem analyses and incorporated the following drug categories: cannabinoids, amphetamines, cocaine and metabolites, benzodiazepines, Z drugs, opioids, anticonvulsants, first generation antihistamines, muscle relaxants, dissociatives, hallucinogens, barbiturates, and miscellaneous drugs (see table).

Conclusion/Discussion:

This sensitive, rapid and multi-class method increases the scope of the SFOCME's previous testing regime in order to meet and exceed all national guidelines and eliminates blind spots, while reducing the number of tests required at the SFOCME to just one. The extraction is achieved by protein precipitation followed by size-exclusion filtration, which eliminates the need for more time and resource consuming extraction methods. The described developed and validated method is, to the authors' best knowledge, the only single procedure to meet all national DUID recommended target drugs and/or metabolites. Additionally, the method has been validated for use in other forensic casework such as drug facilitated crime and death investigation postmortem casework.

Analyzing drugs in neonates using oral fluid and urine to develop a prediction model for neonatal abstinence syndrome and assess the implications in forensic toxicology

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Background/Introduction:

Neonatal drug exposure is currently assessed using meconium, urine, blood, hair, or umbilical cord tissue/blood. Due to the invasiveness, challenges and limitations of collection, and/or analytical difficulties of these matrices, oral fluid may be a more desirable matrix in diagnosing opioid exposure and risk for withdrawal in neonatal abstinence syndrome (NAS). As a consequence of the large size and potential chemicals, traditional oral fluid collection devices are not viable options. Unstimulated and stimulated infant oral fluid samples have been used for therapeutic drug monitoring as an alternative matrix to blood.

Objectives:

The objective of this study was to assess the ability to detect drugs related to NAS in neonatal oral fluid using an ad-hoc collection device and urine to assess opioid exposure and risk for withdrawal prior to the onset of symptoms.

Methods:

Oral fluid and urine samples were collected under Virginia Commonwealth University's Institutional Review Board from ten neonates born to mothers in methadone or buprenorphine treatment programs. Oral fluid samples were collected by rolling three foam-tipped swabs along the mouth, inner cheeks, and tongue until saturated. The three swabs containing an estimated total volume of 300 µL oral fluid were placed in 2.0 mL of phosphate-buffered saline, centrifuged, and analyzed by ultra-high-pressure liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) for 26 drugs and/or metabolites after solid-phase extraction (SPE) with OFXQ columns. Urine samples were expressed from cotton balls placed inside the neonates' diapers and were analyzed by gas chromatography-mass spectrometry (GC-MS) in scan mode after liquid-liquid extraction (LLE) for basic drugs. Urine samples were also analyzed by UPLC-MS/MS for quantitation. Results were assessed against maternal history and hospital treatment provided to the neonate.

Results:

The following analytes were identified only in urine samples: 2-hydroxyibuprofen, bupivacaine, caffeine, delta-9-tetrahydrocannabinol, diphenhydramine, fentanyl, lidocaine, norbuprenorphine, nordiphenhydramine, norlidocaine, n-desmethyltramadol, o-desmethyltramadol, pheniramine, and tramadol. The following analytes were detected in both urine and oral fluid: 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline (EMDP), 2-ethylidene-1,5-dimethyl -3,3-diphenylpyrrolidine (EDDP), amphetamine, cocaine, methadone, methamphetamine, nicotine, and norfentanyl. The following analytes were identified only in oral fluid samples: 6-acetylmorphine, benzoylecgonine, cotinine, ethyl sulfate, and morphine. Maternal history and infant medications were reported for 9 of 10 neonates. Additional analytes identified that were not consistent with patient histories, included 6-acetylmorphine, amphetamines, benzoylecgonine, caffeine, diphenhydramine, ethyl sulfate, nicotine, and tramadol. Bupivacaine, fentanyl, lidocaine, norfentanyl, and norlidocaine were consistent with epidurals given during birth.

Conclusion/Discussion:

Three of the ten neonates were treated for withdrawal symptoms by an oral suspension of methadone given twice daily. These neonates were released from the hospital on day of life 18, 20, or 24. Prior to treatment, nine to eleven drugs and/or metabolites were identified in the three neonates, including 2-hydroxyibuprofen, 6-acetylmorphine, amphetamine, benzoylecgonine, bupivacaine, caffeine, cocaine, cotinine, EDDP, EMDP, ethyl sulfate, fentanyl, lidocaine, methadone,

morphine, nicotine, and/or norfentanyl. In two neonates, methadone oral fluid concentrations increased in the first few days of life then significantly decreased in concentration prior to treatment. No methadone was detected in the third neonate, but a variety of other drugs were identified. None of the other neonates presented symptoms of withdrawal and were released from the hospital on day 3 or 4 of life, except for one neonate born pre-term (35 weeks gestation) who was not released until day of life 9. Identifying trends between neonates who demonstrate withdrawal symptoms and those who do not could be important to developing a predictive model for the determination of NAS. Additionally, non-invasive oral fluid collection can be used for screening neonates and infants for forensic and clinical drug testing. When analyzing neonatal samples, complete clinical histories of mother and neonate are necessary to prevent the misinterpretation of data, which could lead to unwarranted criminal or legal complications.

The SOFT Professional Mentoring Program: The Inaugural Year

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Background/Introduction:

The value of an organization investing in a mentoring program is ubiquitously demonstrated for the mentees, mentors, and the organization itself. The mentees become a better-trained and more engaged workforce. Mentors feel more valued when they share experiences while guiding a young employee and value learning new skills, thinking about problems differently, and engaging in a positive activity. The organization benefits by having less talent turnover and an improved work culture while building future managers and leaders. Professional organizations that engage in professional mentoring become an asset to the professional communities they serve.

Objectives:

The purpose of this committee was to develop a sustainable professional mentoring program for SOFT. that could be facilitated by a committee of SOFT members.

Methods:

A Mentoring Task Group was established in December 2018 to assess the value and viability of a professional mentoring program managed by and for SOFT. The committee assessed peer-reviewed literature, met with mentoring and leadership professionals, and reviewed textbook and websites for best practices, viable strategies, and high impact activities. Twelve immediate benefits for SOFT and registered participants were identified. A program was launched in conjunction with the Young Forensic Toxicologists (YFT) Symposium in San Antonio in 2019, with a call for registrants to become mentors, mentees, or both. Registrants were paired and signed a mutually agreed upon contract with goals and outcomes. A year-long program includes an optional breakfast at AAFS, a required webinar training, and a recognition ceremony at the conclusion of the year. A resource page has been developed on the SOFT website. After every activity, a survey was conducted to adjust the program as necessary and improve future programming.

Results:

Of the 12 identified benefits, SOFT would be a beneficiary of 10, mentors of 10, and mentees of 11. Approximately 130 people participated in the group mentoring activity at the YFT. Seventy-four people registered for the mentoring program, with 28 as mentors, 26 as mentees, and 23 who could be a mentor and also wanted to be a mentee, resulting in 41 pairs. Mentors and mentees were paired, based on career progression. Mutually agreed upon contracts defined communication expectations, goals, and projected outcomes. Identified activities ranged from increasing engagement in SOFT to writing publications and presenting at a conference. The mentoring breakfast was sponsored by SOFT and attended by 32 people, and the webinar was attended in-person by 64 participants and has been viewed 19 times. Surveys have already indicated >95% of the participants are satisfied or very satisfied with activities.

Conclusion/Discussion:

A mentoring program with participants from different backgrounds, geographic locations, career aspirations, and sub-discipline specific career paths has highest value and success when diversity is leveraged in mentor-mentee pairings. Therefore, pairings were based on career progress, focusing on general career experience in a pairing, as opposed to geography, age, and sub-disciplines. The organization demonstrated investment in professional development by sponsoring the breakfast, which was highly valued by those who attended. The webinar provided a high impact strategy for mentors and mentees to engage in transparent, productive conversations. The activities identified in the contracts were aspirational, challenging, and forward-thinking as goals which will help them to advance their careers and will support and

advance forensic toxicology. Strategies for improving the program for subsequent years have been identified as activities are assessed. As a result, the mission of building a sustainable program to (i) develop and nurture future leaders of the organization, (ii) provide a forum for one-on-one career advice, and (iii) provide a forum for mutually beneficial transfer of knowledge to support and advance the organization and the forensic toxicology practice is successfully underway.

Keeping Current with Ever-Changing NPS Landscapes – Evaluating Trends and Connecting Case Histories

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Background/Introduction:

The emergence and proliferation of novel psychoactive substances (NPS) continue to challenge forensic toxicologists, drug recognition experts, medical examiners, and coroners. The frequency of NPS involvement in forensic investigations is relatively low compared to cases involving traditional drugs of abuse, however, investigations involving NPS are typically more time consuming and analytically complex. Not maintaining a current scope for NPS testing can result in unexplained impairment or autopsy findings, in turn leading to the possibility of inaccurate or under reporting.

Objectives:

The 2020 NPS landscape looks different that that from 2019 and before. The goal of our program (www.npsdiscovery.org) is to target the early appearance of emerging NPS in the United States and to subsequently monitor positivity and prevalence through an extensive battery of continually updated analytical workflows. Results and new information are then culminated and widely disseminated to stakeholders. This approach allows for evaluation of risk by examining NPS identifications, concentrations, and drug combinations to create the most comprehensive picture of use patterns.

Methods:

Biological samples (or sample extracts) from forensic and clinical toxicology investigations with suspicion of NPS use or ingestion were submitted for testing. Samples were prepared by liquid-liquid extraction (LLE) or solid phase extraction (SPE), depending on the non-targeted or targeted nature of the assay. Identifications of NPS, drugs of abuse, and other relevant substances were conducted using a SCIEX TripleTOF® 5600+ quadrupole time-of-flight mass spectrometer coupled with a Shimadzu Nexera XR ultra high performance liquid chromatograph (LC-QTOF-MS). Quantitative confirmations were conducted using a Waters Xevo TQ-S micro tandem mass spectrometer coupled with a Waters Acquity ultra performance liquid chromatograph (LC-MS/MS).

Results:

The majority of NPS identified through May 2020 resulted from postmortem investigations. Isotonitazene was the most commonly encountered NPS opioid, commonly found in combination with piperidylthiambutene and NPS benzodiazepines. 2-Methyl AP-237 was quantitatively confirmed in two drug overdose cases, fatal and non-fatal, but a marked difference in concentrations was noted (e.g. 35 ng/mL vs. 5,800 ng/mL, respectively). 5F-MDMB-PICA was the most commonly encountered NPS synthetic cannabinoid, however, the prevalence of MDMB-4en-PINACA continued to increase both in combination with 5F-MDMB-PICA and/or 4F-MDMB-BINACA and absent of other synthetic cannabinoids. Flualprazolam positivity continued to increase among NPS benzodiazepines, however, etizolam remained the most prevalent substance in this class. Eutylone largely displaced previously prevalent NPS stimulants, but other stimulants (e.g. N-ethyl pentedrone) were also commonly encountered among this very diverse class. NPS hallucinogen identifications (e.g. MeO-PCP) were the least commonly encountered among all classes.

Conclusion/Discussion:

The NPS opioid landscape has evolved significantly over the last year and since the Drug Enforcement Administration (DEA) enacted core structure scheduling of fentanyl in 2018 to combat the proliferation of new synthetic analogues. Fentanyl analogues have largely disappeared, replaced by the emergence of new non-fentanyl derived synthetic opioids. The most common synthetic cannabinoids have remained consistent over the last year, however, the turnover of second tier substances remains an area of concern for forensic toxicologists. The diversity of NPS benzodiazepines remained low, but recent activity suggested that this market could expand in future months. NPS benzodiazepine combinations with opioids appeared to be increasing in frequency and remain an area of concern as their combined use can result in death. The combined NPS stimulant and NPS hallucinogen markets are the most diverse in terms of number of substances, but

their turnover is typically dominated by a singular substance. Forensic toxicologists must remain aware of trends in the NPS landscape and consider further testing when case histories do not match analytical findings. The use of continually updated analytical workflows should be explored.

Validation of a Method, Sample Analysis, and Determination of the Whole Blood Partition Coefficient for Brodifacoum in Forensic Toxicology Casework using Ultra-High Performance Liquid Chromatography Tandem Mass Spectrometry (UPLC-MS/MS)

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Background/Introduction:

Superwarfarins, in particular brodifacoum, emerged in the forensic toxicology world as a result of adulterated synthetic cannabinoids. While patient symptoms helped to illuminate the class of drugs, the treatment process for individuals that have ingested superwarfarins is intensive. If treatment is stopped too soon, patients will revert to experiencing previous harmful side effects. Plasma is the preferred matrix in a clinical setting, however, measuring the concentration of an analyte in plasma may give misleading information about the total amount of drug remaining in the system when the whole blood partition coefficient is unknown.

Objectives:

The objective of this research was to develop and validate a method to quantify brodifacoum by UPLC-MS/MS, verify the validity of the assay by comparison to concentrations reported by other analytical laboratories, and finally determine the partition coefficient by measuring brodifacoum concentrations in vitro in the plasma and red blood cell layer.

Methods:

Samples were extracted using acetonitrile for protein precipitation and the resulting supernatant was mixed with n-butyl chloride for a liquid-liquid extraction. The organic layer was dried down and then reconstituted in methanol for analysis. The UPLC-MS/MS analysis was carried out using a Waters Acquity TQS system, utilizing a Waters BEH C18 (50 x 2.1 mm, 1.7µm) column with 0.1% formic acid in water and 0.1% formic acid in methanol as the mobile phase. Twelve de-identified case samples, that had been tested by two other laboratories, were analyzed, and the results were compared to ensure accuracy of the method. The equilibration time for the whole blood partition coefficient was determined by incubating whole blood samples at the HQC (high quality control) and LQC (low quality contronl) levels to ensure that results are not concentration dependent over a three-day time course. Whole blood samples were then incubated for the determined equilibration period and the plasma and red blood cell layer concentrations were determined.

Results:

Method validation met the criteria set in the ASB Standard 036: Standard Practices for Method Validation in Forensic Toxicology, with the exception of ion enhancement. A linear assay range was established from 10 - 250 ng/mL. For the twelve cases, nine initially agreed with previously reported results and three showed a lower concentration. These cases were re-tested with standard addition to investigate if the differences were due to matrix effects between different patients. Ultimately, samples assayed were able to demonstrate reproducible results. The following table shows the inter-lab comparison and the results using standard addition.

Conclusion/Discussion:

A quantitative method for the analysis of brodifacoum in human whole blood was successfully validated and results were consistent with reported concentrations from other laboratories. Samples reanalyzed with standard addition indicated that there may be nuances between interpatient samples during analysis, thus, standard addition would be the best form of quantitative analysis for suspected brodifacoum samples. While it is not feasible to determine all of the pharmacokinetic and pharmacodynamic properties of all new psychoactive substances, there is a need for properties to be further studied, as illustrated by brodifacoum. Ultimately, constant vigilance is needed with clinical cases in order for toxicological research to fill the gaps between analysis results and the proper treatment plan.

SOFTEMBER PLATFORM ABSTRACTS SESSION 2

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Chiral Separation and Quantitation of Methylphenidate, Ethylphenidate and Ritalinic Acid using Supercritical Fluid Chromatography

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Background/Introduction:

Supercritical fluid chromatography (SFC) utilizes a fluid with properties between a liquid and gas, most commonly CO2 due to solvent strength and lack of toxicity. SFC has proven to be an efficient way to separate enantiomers. Methylphenidate (MPH) is commonly prescribed to treat ADHD. MPH metabolizes to ritalinic acid (RA) and if co-ingested with ethanol, also to ethylphenidate (EPH). MPH, EPH and RA are chiral compounds with two chiral centers, giving rise to threo- and erythro-isomer pairs. MPH is prescribed as d/l-threo with dextro providing the pharmacological effects. Due to the difference in activity, chiral separation is necessary to interpret results. To date, there are no SFC methods for separating MPH and metabolite enantiomers.

Objectives:

The goal of this study was to optimize and validate a method for the quantification of d/l enantiomers of threo-MPH, EPH and RA in postmortem blood using SFC coupled with mass spectrometry.

Methods:

Methylphenidate, ethylphenidate and ritalinic acid were extracted from blood (250µL) using solid phase extraction. Calibrators were prepared by fortifying 25µL appropriate stock solution to result in 0.25, 0.75, 2.5, 7.5, and 25ng/mL in blood for MPH and EPH and 10, 30, 100, 300, and 1000ng/mL in blood for RA. Mixed internal standard solution (25µL) was added to calibrators, controls, and cases. Samples were buffered (phosphate buffer pH 6, 100mM) then centrifuged and loaded onto an Agilent Bond Elut (130 mg) cartridge conditioned with methanol and phosphate buffer. Following washes with 0.1M acetic acid and methanol, compounds were eluted with 2% ammonium hydroxide in methanol. Analysis was performed on an Agilent Technologies 1260 Infinity SFC Control Module with an Agilent 1260 Infinity Liquid Chromatograph coupled to an Agilent Ultivo Triple Quadrupole. Isocratic separation was achieved in 4.5min (1.8 mL/min) using 12% trifluoroacetic acid (0.2%) in supercritical CO2 on an Agilent Chiral-V column (2.7um, 2.1x100mm, 20°C), followed by wash and re-equilibration for a run time of 7.5min. Using 0.3mL/min of 0.1% formic acid in MeOH:diH2O (85:15) post-column addition analytes were detected using multiple reaction monitoring with two transitions. Linearity, bias and precision, limit of quantification, recovery and matrix effects were assessed. As proof of concept, forty-nine postmortem blood specimens were analyzed and compared to results from an achiral liquid chromatography tandem mass spectrometry (LCMS) method.

Results:

Linear ranges for d,l-MPH and d,l-EPH were 0.25-250ng/mL, and 10-1000ng/mL for d,l-RA (R2 >0.99 for 5 days). Matrix effects were -8-11% except for RA with 25-27% ion suppression. Extraction recovery was >79%. Bias was -8-0.8% for all analytes with maximum within-day and between-day precision of 15% and 12%, respectively. Results from postmortem samples are summarized below (see attached table). Total RA concentrations (d+l) were on average within 7.3% of those reported by achiral LCMS method. On average, total EPH and MPH concentrations were 36 and 55% lower than original concentrations, respectively. All EPH cases were positive for MPH, indicating ethanol co-ingestion. For 11 cases with <5ng/mL MPH by LCMS, no enantiomers were detected with this SFC method. Analyte loss after achiral analysis was time- and concentration-dependent.

Conclusion/Discussion:

This method was developed and validated for the quantification of d,l-methylphenidate, d,l-ethylphenidate and d,l-ritalinic acid utilizing SFC-MS. When compared to an achiral technique, this method demonstrated good agreement for RA, but lower concentrations for MPH, which may be explained by instability. Concentration ranges may indicate abuse when above reported therapeutic ranges. Though MPH is prescribed as a racemic mixture, the ratios of d:l may change over time providing insight into time of intake. To our knowledge, this is the first method to use SFC-MS to separate and quantify these enantiomers in a single assay without the need for chiral derivatization.

Ethanol bioavailability demonstrated by dose and particle size analysis of aerosols generated using ethanol-containing e-liquids

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Background/Introduction:

Electronic cigarettes (e-cigs) were created as an alternative nicotine delivery system for traditional combustion cigarettes. E-liquids are typically composed of nicotine, flavoring agents, propylene glycol, and vegetable glycerin. An aerosol is produced by the electronic cigarette when the e-liquid passes over the heated coil, vaporizes, and then condenses with water in the atmosphere. The size of the droplets, called particles, formed in the aerosol can vary and is a major factor in determining where and if that particle will deposit in the lung. Small particles can penetrate deeper into the alveoli of the lung tissue and increase their probability of being absorbed into the bloodstream. Ethanol is used as a solvent for flavoring chemicals and to dissolve solid tablets and pills and decrease e-liquid viscosity. As an often unlabeled ingredient in e-liquid formulations, vaping ethanol can potentially interfere with and mislead forensic ethanol analyses.

Objectives:

The purpose of this experiment was to evaluate the dose of ethanol in a puff and the ethanol-based aerosol particle size profile to determine if it can be effectively delivered into the deep lung by an e-cigarette.

Methods:

Ethanol e-liquids (50:50 PG:VG) were prepared at four different concentrations (5, 10, 15, 20%). For dose capture analysis, the e-liquids were aerosolized using a KangerTech AeroTank e-cigarette with a 1.8Ω pre-assembled atomizer, and an eGo-V2 variable voltage battery kept at a constant voltage of 4.3 V. Each sample consisted of four 4 sec “puffs” at 2.3 L/min, and was replicated five times for each ethanol concentration. The aerosol produced was collected using a water trap, and the water from this trap was analyzed using a Shimadzu GC 2030 with HS-20 headspace attachment (HS-GC-FID). For particle size analysis, the 10% ethanol e-liquid was aerosolized onto a 10 stage Micro-Orfice Uniform Deposit Impactor (MOUDI). Each sample consisted of ten 10 sec pulls at 30 L/min. The MOUDI stages feature holes that vary in size per each stage to represent a particular particle size range (0.056 to 18 m). These ranges correlate to where in the pulmonary tract particles are able to travel to. Particles that cannot flow through the holes will land on the stage, which is weighed before and after the 10 burns to determine which size range most of the aerosol particles fall into. The stages were then rinsed with propanol and analyzed using the HS-GC-FID.

Results:

Ethanol concentrations ranged from 8.41 – 4080 µg per puff, and varied based on percentage of ethanol in the e-liquid formulation. More than 90% of the ethanol was deposited within the particle size range of 0.172 – 0.54 µm, and the mean mass diameter (MMAD) was determined to be 0.38 ± 0.08 µm.

Conclusion/Discussion:

This study highlights the ability of e-cigs to deliver ethanol. Ethanol concentrations yielded from dose capture analysis show that ethanol is a constituent of the condensation aerosol. The particle size profile indicates that ethanol is effectively deposited into the alveoli of the deep lung tissue for absorption into the bloodstream. The immediate effects of inhaling ethanol are currently unknown. However, the implications for forensic analyses, including the interpretation of ethanol metabolites in urine as ethanol oral consumption, are unknown.

Evaluation of multiple methods for the analysis of cannabinoid infused beverages.

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Background/Introduction:

In just over a year since the December 2018 passing of the Agriculture Improvement Act (Farm Bill) legalizing hemp, use of cannabinoids in the United States has increased tremendously. The increase has been primarily in marijuana infused products. Initially as food products, and now as beverages. Many of these products are reported not to contain the psychoactive drug delta-9-tetrahydrocannabinol (THC), but are reported to contain cannabidiol (CBD), and other cannabinoids, and are sold because of their reputed medical and recreational properties. Regulation of these beverages is very limited, and only if they contain THC, is there regulation in the states where THC is legal. Thus, there are limited regulations on the actual formulation of these beverages, and no standardization of methods for content and potency. There is an increased need for accurate methods to determine THC and CBD content in these beverages. The three most common beverage matrices involve fermented (beer/ale), brewed (tea/coffee), and high sugar (soft drinks). Each has issues, and since most beverages contain mixed matrices, it is not advisable to try to analyze beverages as a single class, without validation of each matrix. Many of these beverages contain plant materials and sugar, so a simple dilution method is also not advisable.

Objectives:

To develop a single method for the analysis cannabinoids (THC, CBD and CBN) in three different cannabinoid infused beverage matrices (high sugar, brewed and fermented).

Methods:

Multiple methods (40+) were evaluated for process efficiency (%PE). These included simple dilutions, rapid solid phase extraction (SPE) and quick easy cheap effective rugged safe (QuEChERS) methods, with various solvents, substitutions and buffering. Cannabinoids have a very low solubility in aqueous solvents, so the cannabinoids had to be solubilized in an emulsion (surfactant (2% fruit pectin), carrier oil (12.6 % canola oil) and beverage (85.40%)). %PE was determined at 14 mcg/mL (n=3) for each extraction method. The %PE was determined using the post-extraction addition method. An ideal method had a %PE within 75 – 125 % for all three matrices. An acceptable method had %PE variation in all three matrices of <± 15%. Samples analysis was performed using a previously presented and published UPLCMSMS method.

Results:

The %PE was similar in the fermented and brewed matrices in most methods, however the %PE was higher (20 – 50%) in the sugar matrix. Simple dilutions with solvents did not adequately remove plant materials for UPLCMSMS analysis. The only acceptable method used the United Chemical Technologies Clean Screen FASt/THC 200mg/3mL (UCT THC) column. %PEs for fermented, brewed and sugar matrices were 40, 45, and 54%, respectively. Sample preparation involved mixing 25 mcL of beverage, 225 mcL water and 250 mcL acetonitrile, then adding to the column and eluting with 80 psi of air before analysis.

Conclusion/Discussion:

While no extraction method was determined to be ideal, UCT THC column provided acceptable results.

Toxicological Findings in a Large Population of Drug Facilitated Crime Cases using Accurate Mass Screening

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Background/Introduction:

This presentation reports toxicological data from 1576 urine and 1102 blood cases submitted to NMS labs for analysis in cases of drug facilitated crimes (DFC) between June 2018 – February 2020.

Objectives:

To examine the incidence of drugs detected in DFC cases and compare commonly observed combinations of drugs.

Methods:

Beginning in June 2018, drug screening in DFC cases was modified to include LC-MS/MS accurate mass (time of flight) for most analytes while continuing to use ELISA-screening for cannabinoids and barbiturates, GC/MS testing for gamma-hydroxybutyrate (GHB) and dual column headspace gas chromatography with flame ionization detection for volatiles. For urine, samples were hydrolyzed with beta-glucuronidase prior to accurate mass analysis. Presumptive positive findings were confirmed using a variety of analytical methods and evaluated for trends in drugs of abuse and prescribed drugs. The analytes and reporting limits in the scope of testing meet or exceed most of the proposed Academy Standard Board recommendations for forensic toxicology urine testing in drug-facilitated crime investigations.

Results:

Of all 2678 samples submitted for testing, there were 736 cases which included paired blood and urine samples; however data analysis for each matrix was considered independently. The most common findings in blood and urine samples (including metabolites) are summarized in Table 1. <Insert Table 1, attached file>

Conclusion/Discussion:

As expected, the ability to detect any analyte (including metabolites) was greater in urine (3684 analytes in 1576 samples) than for blood (1914 analytes in 1102 samples). 272 (17.3%) of urine samples were positive for 5 or more analytes compared with 80 (7.26%) for blood. None detected cases for urine were 24.0% of samples, compared with 33.0% for blood. Several observations were notable. In blood only 7 cases were positive for acetone while 204 cases were positive in urine. This is consistent with the increased likelihood for detectable ketones in urine (whether produced in vivo or in vitro). Overall, excluding volatiles and cannabinoids, the most common drug class detected was stimulants (25.0% of analytes detected). Of these, 57.3% were detected in cases collected in California (which represent only 35.1% of submitted specimens) demonstrating an expected regional bias for stimulant drugs in submissions from that state. After stimulant drugs, the most common drug classes detected (as a percent of analytes) included benzodiazepines (10.8%), antidepressants (8.99%), opioids (8.70%), and antihistamines (3.26%). For those drugs often associated with “date rape” GHB was detected in 10 blood samples (3.9 – 81 mcg/mL, median 16.5 mcg/mL) and 42 urine samples. Of these, 25 urine samples were GHB-positive at <5.0 mcg/mL likely representing endogenous GHB. The GHB cutoff was later changed to 5.0 mcg/mL. There were 17 urine cases that were positive for GHB at >5.0 mcg/mL (5.6 – 2200 mcg/mL, median 83 mcg/mL). Tetrahydrozoline (THZ) was detected in urine in 33 cases (0.058 – 80 ng/mL, median 0.75 ng/mL) but only in 1 blood case (0.18 ng/mL). This happened to be the same case with 80 ng/mL in urine suggesting a blood cutoff of <0.050 ng/mL is needed for THZ. Finally, there was one case for which blood and urine were submitted that was positive for flunitrazepam (4.2 ng/mL, blood), 7-aminoflunitrazepam (7.5 ng/mL, blood; 330 ng/mL, urine) and norflunitrazepam (2.7 ng/mL, blood; 2.3 ng/mL urine). This case was also positive for cannabinoids. The most common drug classes in combination were stimulants and cannabinoids (Blood -91 cases, 8.26%; Urine – 201 cases, 12.7%). followed by volatiles (acetone excluded) and cannabinoids (Blood – 54 cases, 4.90%; Urine – 132 cases, 8.38%). Excluding volatiles, stimulants paired with either an opioid, benzodiazepine or antidepressant were the next most commonly encountered combinations for both blood and urine. Since 736 cases were paired blood and urine, some bias could be introduced as a result. In a study

of a large number of drug-facilitated crime cases, results demonstrate that the analytes detected with the highest prevalence are not the typical “date rape drugs,” but are instead drugs that are frequently seen in other types of cases, such as postmortem and DUID cases. Caveats to the findings in this study are not knowing the elapsed time between the alleged crime and the time of specimen collection. Also, the history of any drugs abused and/or prescribed are not known.

Laboratory modernization to address CA state toxicology needs: Whole blood, urine and oral fluid analysis by LC-MS/MS coupled with the novel Unispray™ ionization source

Authors and Affiliations: *Daniel Ayala[1], Daniel Williams[1], Jirair Gevorkyan[1], Kristen Burke[1] [1]California Department of Justice, Bureau of Forensic Services, Toxicology Laboratory

Background/Introduction:

The California Department of Justice (DOJ) Bureau of Forensic Services (BFS) toxicology laboratory provides testing for 45 out of 58 California counties. BFS receives over 5100 samples on average each year for analysis, and has observed a rise in drug cases over the last two decades. Prior methodology employed by BFS primarily consist of GC-MS. Unfortunately, the laboratory systems have been stressed by increase in polypharmacy, novel psychoactive substances, proliferation of the opioid epidemic, and availability of cannabis and cannabis products after the promulgation of cannabis law marked by California Proposition 64 in 2016. These challenges prompted BFS to develop and validate methodology using modern instrumentation that will evaluate smaller volumes of sample for a larger scope of drugs with greater sensitivity and decreased turnaround time.

Objectives:

Develop and validate methodology that will consolidate and expand laboratory scope. Specifically, two methods that accommodate for routinely encountered anticonvulsants, antidepressants, benzodiazepines, blockers, cannabinoids, dissociatives, hallucinogens, metabolites, muscle relaxers, opioids, stimulants and Z-drugs developed and validated according to the American Standards Board (ASB) standard 063 guidelines. Sample matrix includes blood, urine and oral fluid to address analytical challenges (drug stability, delay of blood draw after driving, matrix availability and invasiveness of collection). In addition, the methodology is to fit with the ISO 17025 laboratory workflow.

Methods:

Sample preparation consists of initial acetonitrile crash extraction and either 1) centrifugation and dilution with initial LC conditions or 2) solid phase extraction (SPE) followed by dry down and reconstitution into initial LC conditions. Method #1 evaluates 56 non-cannabinoid analytes in 100 µL of blood using preparation #1, or oral fluid and urine using preparation #2. Method #2 evaluates 6 cannabinoids in 500 µL of whole blood , urine or oral fluid using preparation #2. Chromatographic and mass spectral resolution for targeted analysis are performed on a Waters Acquity ultra high pressure liquid chromatography system fitted with a Waters Acquity BEH C18 column and coupled to a Waters Xevo TQ-XS triple quadrupole mass spectrometer with a Unispray ionization source. The data processing and reporting logistics are designed using Targetlynx and Microsoft Excel Power Query.

Results:

The Waters Xevo TQ-XS triple quadrupole mass spectrometer with a Unispray ionization source has been used to evaluate bias, precision, calibration model, carryover, interference studies, ion suppression and enhancement, limits of detection and quantitation, dilution integrity and processed sample stability. Method #1 validation is complete and in review. Method #2 is undergoing further validation. The processing and reporting power query are in review and planned for LIMS (JusticeTrax) integration.

Conclusion/Discussion:

The evolving nature of modern drug use is a taxing challenge and has required the CA DOJ BFS toxicology laboratory to adapt in order to meet the demands of the jurisdictions it serves. The laboratory has adopted methodology to confirm and quantify routinely encountered drugs at low to sub-nanogram per milliliter levels in blood, urine and oral fluid. The expanded scope and faster turnaround that these methods will bring are an essential component of the toxicology laboratory's mission and will facilitate better characterization of statewide drug prevalence in the DUID and DFSA/DFC population.

SOFTEMBER PLATFORM ABSTRACTS SESSION 3

10-year study of fentanyl in Driving Under the Influence of Drugs (DUID) casework

Authors and Affiliations: Ayako Chan-Hosokawa1*, Jolene Bierly1, Barry K. Logan1,2 1 NMS Labs, Horsham, PA 2 Center for Forensic Science Research and Education, Willow Grove, PA

Background/Introduction:

Prior to 2017, heroin, oxycodone and hydrocodone were the most prevalent opioids implicated in DUID investigation cases and fentanyl was rarely tested for. Once illicit fentanyl became commonly available, fentanyl has become the most frequently identified opioid in DUID cases with many suspected heroin cases turning out to be fentanyl positive. This presentation will illustrate the importance of including fentanyl in a routine blood DUID panel highlighting its prevalence and change in concentration over a 10-year period.

Objectives:

To demonstrate the blood concentrations and prevalence of fentanyl and the concomitant findings in DUID investigation cases reported.

Methods:

Data was examined in suspected impaired driving cases received between January 2010 and December 2019 where the blood confirmed positive and was quantitated for fentanyl using an LC-MS/MS analysis with an LOQ of 0.10ng/mL. In our laboratory prior to January 2018, fentanyl was screened using LC-TOF in approximately 13% of DUID cases where a comprehensive screen targeting therapeutic and abused drugs was conducted. In January 2018, fentanyl was added to the routine ELISA screen consisting of 15 drug classes for blood.

Results:

Of 134,853 blood cases examined for DUID over ten years, fentanyl confirmed positive in 4,462 (3.3%) cases, however there were significant changes in positivity over time. Statistical information for fentanyl is summarized below by year in Table 1. In the last four years, the median age of fentanyl positive drivers was between 31 and 33 and gender distribution showed three times more males than females were involved, when information was provided. <Table 1 here - MS Word attached> Table 1: The total number of reported fentanyl positives, %positivity, mean±SD, median, and maximum blood concentration Of 3,659 confirmed fentanyl positive cases in 2018 and 2019, where a general 15 ELISA panel was performed, blood concentrations greater than 4.0ng/mL were observed in 43% (2018) and 54% (2019) of cases. Concentrations less than 1.0ng/mL were seen in 18% of cases and were the most frequently reported concentrations, followed by >10-25ng/mL. In the same dataset, norfentanyl and acetylfentanyl were also reported in >90% and 19% of cases, respectively. When both fentanyl and norfentanyl were positive (n=3,316), the ratio of fentanyl to norfentanyl concentrations was over 2 in 63% of the cases. Fentanyl/norfentanyl was the only finding in 485 cases (13%). By looking at the compound level, 2 and 3 additional compounds were commonly present along with fentanyl in 41% of combined cases. Morphine was present with the highest prevalence at 39%, followed by delta-9 carboxy THC (33%); 6-monoacetylmorphine (6-mam) was reported in <6% of fentanyl positive cases. When analyzed by drug class, 35% of the cases showed one other drug class: stimulants (35%), opioids (29%), cannabinoids (20%) and benzodiazepines (10%). In cases with more than two additional drug classes, opioid was the most frequently report concomitant class.

Conclusion/Discussion:

The average prevalence of fentanyl in drivers in 2018 and 2019 was 9.5% after the addition of fentanyl to the routine DUID drug screen. The median, mean, and highest blood concentrations reported are increasing. Polypharmacy was common; 87% of blood sample was positive for fentanyl and at least one other compound. For cases with one additional drug class, stimulants were the most commonly detected class. Of fentanyl confirmed cases, low prevalence of 6-mam supports the need to test for fentanyl in all heroin suspected cases. Additionally, the concentration of fentanyl, ratio of fentanyl to norfentanyl and the presence of acetylfentanyl can add a value to interpretation in order to aid determination of therapeutic vs. illicit fentanyl use.

Drug driving in England and Wales

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Background/Introduction:

In the United Kingdom, the Road Safety Act 1967 introduced the first legally enforceable maximum blood alcohol level for drivers in the UK and it was no longer necessary for the police to prove impairment of the driver from alcohol consumption. However, if the motorist was thought to be unfit to drive due to drugs, impairment had to be proved. In March 2015, the law in England and Wales was amended to include the offence of driving with a specified drug in the body, if the proportion of the drug in that person's blood or urine exceeded the specified limit for that drug. Seventeen drugs were listed in Section 5A of the Road Traffic Act 1988 with specific limits set in whole blood only (tinyurl.com/ycx3qm8p). The compounds, were considered in two groups, nine with well recognised medicinal uses; clonazepam, diazepam, flunitrazepam, lorazepam, oxazepam, temazepam, amfetamine, methadone and morphine. The remaining six, and two primary metabolites, are compounds that may arise from recreational use; cocaine, benzoylecgonine (BZE), delta-9-tetrahydrocannabinol (THC), lysergic acid diethylamide (LSD), ketamine, methyl-amfetamine, methylene-dioxy-methamfetamine (MDMA), and 6-Mono-acetyl-morphine. The limits for the medicinal drugs were chosen at concentrations likely to cause impairment while those for the recreational compounds were per se levels set at lowest permitted concentrations but above which that may result from accidental exposure.

Objectives:

This abstract presents the drug concentration findings in whole blood of approximately one thousand motorists suspected by the police of committing a Section 5A drug-driving offence in England and Wales.

Methods:

The samples were fluoride-oxalate anti-coagulated whole blood taken at the request of the police. The blood drug concentrations were measured using two analytical methods. THC was quantified using GS-MS/MS, and the remaining sixteen compounds by LC-MS/MS. Both assays were accredited as ISO 17025 compliant by the United Kingdom Accreditation Service (tinyurl.com/yyocdj3h). MS Excel was used to produce summary statistics of the results.

Results:

The median age of the drivers was 26 years and ranged between 16 and 65 years of age with an inter-quartile range of 22 to 32 years. Most of the samples submitted, 92%, were from men. Of the blood samples submitted 18% contained none of the 17 drugs and 11% contained drug concentrations that were below the prescribed limit. 64% of the samples contained one drug above the limit, 6% contained two, 0.8% contained three and 0.1% of blood samples had four drugs over the limit. THC was detected in 78% of the positive samples and cocaine/BZE in 27%. Of those drivers testing positive for two drugs, most were a combination of THC and cocaine/BZE.

Conclusion/Discussion:

As expected, cannabis and cocaine make up the bulk of the positive drug driving samples. Of concern is that 18% of the samples contained none of the 17 drugs yet the drivers must have been considered impaired when stopped by the police. Research is planned to screen these samples for other potentially impairing drugs.

Drugs on Board?: A Study of the Presence of Drugs in Driving Under the Influence Cases Where Only alcohol is Pursued in Northern Virginia

Authors and Affiliations: Courtney M. Wardwell*1, Teri McIntosh1, Kevin Schneider1, Carol L. O'Neal1. 1Virginia Department of Forensic Science, Manassas, VA.

Background/Introduction:

According to the Code of Virginia, a person is considered driving under the influence if the amount of alcohol and/or drugs impairs their ability to operate a motor vehicle. While there are currently increased penalties based on alcohol content, there are no increased penalties for operating a motor vehicle as related to drug presence or concentrations. Therefore, driving under the influence (DUI) cases that have an ethanol above a 0.10% w/v in blood are not tested for the presence of drugs. Any potential additional drugs that could be present are not reported. This study was performed to have a better understanding of the prevalence of co-ingested drugs with alcohol in drivers and estimate the impact performing those analyses would have on the analytical process.

Objectives:

To determine the prevalence of driving under the influence (DUI) of alcohol and drugs in DUIs in Northern Virginia where alcohol was reported, but no drug screening was performed.

Methods:

One hundred DUI cases were selected from those where alcohol was above the administrative stop testing cutoff, which precluded them from drug screening. This sample set included cases from jurisdictions across the Northern Virginia district from May to November 2019. In 2019, the Northern district had 833 DUI cases in which alcohol was above the cutoff, giving this study a sample size of ~12%. There were also 583 DUID cases submitted. The samples were screened for 17 drugs and/or drugs classes by ELISA. The VA-DFS Procedures Manual for drug screening by ELISA (Tecan Freedom Evo, Tecan Hydroflex Washer, Tecan Sunrise Basic Reader) was followed using Immunalysis ELISA kits. The VA-DFS Procedures Manual is available on our website www.dfs.virginia.gov.

Results:

Of the 100 tested DUIs, 44 screened presumptively positive for one or more drug/drug class, and 56 had no presumptive positive pending results. The most commonly seen drug was Cannabiniods (THCA/THC) (22 cases). Based on the number of DUIs where only alcohol was pursued in Northern Virginia in 2019 (833), completing drug screening would require 833 more ELISA samples (~20 batches) with an estimated 367 of those samples continuing on to confirmatory analyses. A summary of these results is in Table 1.

Conclusion/Discussion:

By testing all DUIs for the presence of drugs, there would be a more comprehensive understanding of the number of drivers who are operating a motor vehicle on a combination of alcohol and/or drugs and the types of drugs that are used before driving, whether illicit or prescription. However, by testing all DUIs the average turnaround time for each case will increase with more testing needed. Increased testing would use more resources and increase the number of analyses that need to be completed. For example, if 184 more cases need to be confirmed for Cannabiniods (THCA/THC) in 2019, that would require approximately 6 batches at 2 days per batch, costing the Commonwealth ~$3500 per year just for the analyst's time, not including consumables, lab supplies, instrument costs, etc. Even though more resources would be needed, this additional data would allow for DUI drug trends to be tracked over time. Further assessment needs to be completed to determine the total impact of this data.

Prevalence of Novel Benzodiazepines in DUI cases in Colorado 2018 -2020

Authors and Affiliations: Vanessa Beall, Colorado Bureau of Investigation, Arvada, CO

Background/Introduction:

Benzodiazepines have been prescribed since the 1960s for their sedative properties, to treat anxiety, and are commonly encountered in cases involving driving under the influence (DUI). Over the past several years, novel benzodiazepines have become readily available for purchase on the internet because most are not approved for use by the FDA in the United States, while others may be approved for use in other countries. Novel benzodiazepines have been detected in DUI casework in Colorado with increasing prevalence since 2015.

Objectives:

To determine the prevalence of novel benzodiazepines in DUI casework in Colorado in 2018 – 2020 and to demonstrate how the laboratory handled the emergence of Flualprazolam. DUI case examples with novel benzodiazepines will also be discussed.

Methods:

Blood samples submitted to the laboratory for analysis in DUI cases are screened via ELISA for 14 drug groups to include Benzodiazepines. Benzodiazepine presumptive positive cases are confirmed by solid phase extraction (SPE) and liquid chromatography tandem mass spectrometry (LC/MS/MS). Both methods are validated according to SWG-TOX guidelines. The benzodiazepine confirmation includes quantitative determination of 9 typical benzodiazepines (Alprazolam, Clonazepam, 7-aminoclonazepam, Lorazepam, Diazepam, Nordiazepam, Oxazepam, Temazepam, and Chlordiazepoxide), and the qualitative determination of 22 benzodiazepines (Midazolam, Flunitrazepam, 7-aminoflunitrazepam, Clonazolam, Flubromazepam, Phenazepam, Pyrazolam, Triazolam, Delorazepam, Estazolam, Clobazam, Diclazepam, Flurazepam, Flubromazolam, Etizolam, Nimetazepam, Bromazepam, Adinazolam, Lormetazepam, Demoxepam, Nitrazepam, and Flualprazolam). In January 2020, Flualprazolam was added to the existing benzodiazepine confirmation panel, and validated for qualitative determination. From July 2015 – June 2019, case samples submitted to the laboratory were tested as law enforcement directed for either ethanol/volatiles and/or drugs. In July 2019, the laboratory changed to standardized testing so that all submitted cases are analyzed for ethanol/volatiles and drugs of abuse. Statistical analysis was performed on two time periods, one during non-standardized testing (July 1, 2018 – December 31, 2018) and the other during standardized testing (July 1, 2019 – December 31, 2019) to show the prevalence of typical benzodiazepines versus novel benzodiazepines. Further statistical analysis was performed on data from January 1, 2020 – April 29, 2020 to demonstrate the emergence of Flualprazolam.

Results:

Table 1 shows reported typical vs. novel benzodiazepines for the two 6 month time periods in 2018 and 2019, as well as the first 4 months of 2020. For the 2018, 2019, and 2020 time periods, Flubromazolam, Etizolam, and Clonazolam were the most commonly reported novel benzodiazepines, respectively. Of further note, in January 2020 the laboratory added Flualprazolam to the existing benzodiazepines panel and as of April 2020 it is the second most commonly reported novel benzodiazepine.

Conclusion/Discussion:

At this time, we believe the increased prevalence of novel benzodiazepines in DUI casework in Colorado is due to a combination of the laboratory's comprehensive confirmation panel, the standardized testing protocol, and the increase use of novel benzodiazepines in the DUI population. Therefore, it is recommended that laboratories performing DUI casework include novel benzodiazepines in their confirmation panels and consider a standardized testing protocol. In Colorado, novel benzodiazepines such as Clonazolam and Flualprazolam are being reported as often as common Opioids like Hydrocodone, Methadone, and Tramadol. Finally, it is advised to monitor emerging novel benzodiazepines to determine if they should be qualitatively validated and added to existing panels.

Results from the 2020 Survey for Drug Testing in DUID and Traffic Fatality Investigations

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Background/Introduction:

In 2004 the National Safety Council Alcohol, Drugs and Impairment Division (NSC-ADID) began documenting analytical practices of toxicology laboratories performing driving under the influence of drugs (DUID) case testing. These efforts were designed to gather more information about testing best practices, capabilities and needs, and to identify the most commonly encountered analytes in DUID and motor vehicle fatality cases in order to make standardized recommendations for laboratories performing this type of casework. First published in 2007, the recommendations were updated in 2013 and 2017 after surveying performing laboratories, analyzing their responses, and reviewing changing patterns of drug use. In 2019, the Academy Standards Board (ASB) cited the 2017 recommendations as a basis for their proposed guideline for forensic toxicology testing in impaired driving investigations.

Objectives:

In 2019, the NSC-ADID proposed another survey for the purposes of updating the 2017 guideline document. The objective was to focus specifically on Tier I and Tier II scope of testing and corresponding cutoffs for screening and confirmation, and also to evaluate whether emerging drugs should be added to either scope.

Methods:

The 2017 survey instrument was updated by the NSC-ADID committee, and was sent via SurveyMonkey(TM) to toxicology laboratories in the U.S. and Canada performing testing in DUID and motor vehicle fatality casework. Of 84 laboratories that were sent the survey, 65 completed the survey such that their responses could be included. Laboratories were surveyed on their testing practices, specifically scope of testing, matrices tested, screening and confirmation cutoffs, and compliance with the 2017 recommendations. Questions regarding drug prevalence, laboratory methods, and resource needs were also addressed.

Results:

Of those laboratories who participated, 89% test blood samples, 63% urine samples, and 3% oral fluid samples. The top three screening methods for blood samples were Enzyme-Linked Immuno-Sorbent Assay (ELISA) (51%), Gas Chromatograph-Mass Spectrometry (GC-MS) (35%), and Liquid Chromatography-Mass Spectrometry (LC-MS) (31%). 23% reported using Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) for screening. The top three confirmation methods reported were LC-MS (88%), GC-MS (71%), and LC-HRMS (12%). Note that LC-MS includes LC-MS/MS. A total of 35% of laboratories stated they report unconfirmed screening results under some circumstances. Reasons given for reporting unconfirmed screening results included the type of substance detected, inability to provide confirmation testing, insufficient sample volume, and laboratory policy. For screening practices in blood, between 79% and 100% (mean 95%) of laboratories screened for the Tier I drug classes, and between 56% and 100% (mean 82%) met the recommended screening threshold. The highest rates of compliance were for tramadol, methadone, and diazepam, while the lowest rate was for morphine. For confirmatory practices in blood, between 51% and 95% (mean 85%) of laboratories confirmed for the Tier I drug classes, and between 49% and 98% (mean 80%) met the recommended confirmation threshold. The highest rates of compliance were for benzoylecgonine, carboxy-THC, tramadol, and CNS depressants, while the lowest rate was for buprenorphine. A total of 12% of laboratories met or exceeded all of the 2017 recommendations for con-

firming blood samples, while 40% are currently making changes to methods to meet the recommendations. The top ten most prevalent drugs reported by laboratories in blood were THC and metabolites; methamphetamine; cocaine and metabolites; alprazolam/alpha-hydroxyalprazolam; clonazepam/7-aminoclonazepam; morphine; fentanyl; diazepam/nordiazepam; oxycodone; citalopram. The top three most requested compounds for inclusion in Tier I due to increased prevalence were gabapentin, etizolam, and flualprazolam.

Conclusion/Discussion:

Data from this survey will be critically reviewed and analyzed, in the context of changing patterns of drug use, and updates to the 2017 recommendations will be published in the Journal of Analytical Toxicology. The full survey data are posted at http://www.forensicscienceeducation.org/forensic-research/toxicology/duid/duid-survey/. Future surveys and updates are planned.

SOFTEMBER PLATFORM ABSTRACTS SESSION 4

Society of Forensic Toxicologists Inc.®

7 Year Trend Analysis of Δ9-THC Blood Concentrations in Driving Under the Influence of Drugs Cases in Nassau County, NY from 2013-2019

Authors and Affiliations: David Cook*, Julia Diaz, Stephanie Minero, Timothy Hahn, Joseph Avella Nassau County Medical Examiner, 2251 Hempstead Turnpike, East Meadow, NY 11554

Background/Introduction:

Marijuana contains many different cannabinoids of which Δ9-tetrahydrocannabinol (Δ9-THC) is the primary psychoactive compound and is most responsible for the impairing effects associated with its use (1). Δ9-THC is a lipophilic compound which exerts is psychoactive effect through activation of cannabinoid receptors causing alterations in senses, changes in mood, impaired memory and impaired body movements among other effects (2). According to the CDC approximately 12 million drivers aged 16 and over drove a vehicle under the influence of marijuana in 2018 (3). In recent years the legal status of marijuana has begun to change across the country with medicinal marijuana now being available in 33 states and adult recreational marijuana use now legal in 11 states (4). New York has tried unsuccessfully to legalize recreational marijuana in recent years failing to get the approval of the state Senate. However, the state governor and legislature plan to propose yet another bill in 2020 that would legalize recreational use of marijuana.

Objectives:

The purpose of this study was to analyze historical results from Driving Under the Influence of Drugs (DUID) casework presented to the Nassau County Medical Examiner's office toxicology department to determine what if any trends have occurred in the measured analytical concentration of Δ9-THC and its primary inactive metabolite 11-Nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH) in the blood of tested drivers.

Methods:

Samples submitted by Nassau County law enforcement agencies for analysis in driving under the influence (DUI) were analyzed for drugs beginning January 2013 through December 2019. Cases screening positive via Enzyme Multiplied Immunoassay Technique (EMIT) were quantitated for Δ9-THC and THC-COOH using Gas Chromatography/ Mass Spectrometry (GC/MS) following solid-phase extraction and derivatization. All reported quantitative results from Δ9-THC and THC-COOH cases were sequestered and statistically analyzed to determine the annual means and medians as well as to track the occurrence rate of Δ9-THC values >5 mcg/L. Annual data were then compared using one-way ANOVA to determine statistical significance

Results:

From 2013-2019 the number of samples requested for blood drug analysis by law enforcement increased 199% from 148 in 2013 to 295 in 2019 peaking at 346 in 2017. During this time the percentage of positive Δ9-THC cases varied slightly but no significant trends were observed. However, throughout this time period a statistically significant increase in mean concentration at $p<.05$ was observed for Δ9-THC [$F(1,107) = 5.37$, $p=0.022$] and THC-COOH [$F(1,100) = 4.00$, $p=0.048$]. The mean concentration of Δ9-THC and THC-COOH in 2013 was 3.7 and 151.3 mcg/L with a range of 0.8-13.4 and 10-575 mcg/L respectively (n = 30). The mean concentration of Δ9-THC and THC-COOH in 2019 increased to 6.1 and 236.5 mcg/L with a range of 0.8 – 23.9 and 17.2 – 896.2 mcg/L respectively (n = 79). Perhaps most notably, along with an increase in Δ9-THC concentration there was an observed increase in occurrence of cases with blood concentrations of Δ9-THC >5 mcg/L.

Conclusion/Discussion:

The observed trend shows that while marijuana use in Nassau County drivers may not have significantly increased over the past 7 years, a significant rise in measured Δ9-THC concentrations in the blood of these drivers was observed. Alternative methods of marijuana consumption such as vaping and edibles becoming more mainstream as well as recent trending societal changes in perception and legal status of marijuana may have contributed to these findings and suggests a need for more public awareness on the dangers of driving under the influence of marijuana. References:

1.https://www.drugabuse.gov/publications/drugfacts/marijuana 2.Felder CC, Glass M. Cannabinoid receptors and their endogenous agonists. Annual Review of Pharmacology and Toxicology. 1998;38:179–200. 3.Azofeifa A, Rexach-Guzmán BD, Hagemeyer AN, Rudd RA, Sauber-Schatz EK. Driving Under the Influence of Marijuana and Illicit Drugs Among Persons Aged ≥16 Years — United States, 2018. MMWR Morb Mortal Wkly Rep 2019;68:1153–1157. DOI: http://dx.doi.org/10.15585/mmwr.mm6850a1 4.https://www.ncsl.org/research/health/state-medical-marijuana-laws.aspx

CANNABIDIOL (CBD) DOES NOT CONVERT TO Δ-9-TETRAHYDROCANNABINOL (Δ-9-THC) IN THE BODY BUT THC-CONTAMINATED CBD MAY PRODUCE POSITIVE MARIJUANA DRUG TESTS

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Background/Introduction:

Although CBD is not intoxicating like Δ-9-THC, there are risks associated with its use. The U.S. Agricultural Improvement Act of 2018 (aka, The Farm Bill) removed hemp and its derivative products from the U.S. controlled substances list and allowed hemp to contain ≤0.3% Δ-9-THC. CBD products advertised for their purported health benefits are widely sold in retail outlets and the Internet. Despite their popularity, there is little regulatory oversight of these products and labeled concentrations of CBD and Δ-9-THC are often discrepant from actual concentrations. There are two posited ways in which CBD consumers may be at risk of testing positive for marijuana. First, consuming CBD products that contain legal amounts of Δ9-THC may lead to a positive test. Second, in vitro studies have shown that CBD can convert to Δ-9-THC (and Δ-8-THC) in the presence of acid, leading some to theorize that this conversion may also occur in vivo in the acidic environment of the stomach.

Objectives:

Determine if CBD is converted to Δ9-THC following oral and vaped administration of pure CBD and determine if CBD containing Δ9-THC near the amount allowed by the Farm Bill will produce positive urine tests for Δ-9-THCCOOH.

Methods:

Eighteen healthy, drug-free adults participated in 4 drug sessions separated by > 1 week. Each participant received the following treatments: 1. placebo; 2. pure oral CBD (100 mg); 3. pure vaped CBD (100 mg); 4. vaped CBD (100 mg) contaminated with Δ-9-THC (3.7 mg; 0.39% dry plant weight). Urine, blood and oral fluid samples were collected before and for 58 hours after each session. Urine specimens were screened by immunoassay (DRI Cannabinoid Assay) and hydrolyzed specimens were analyzed for CBD, THC and their respective metabolites by LC/MS/MS.

Results:

Urine specimens from the 18 participants who were administered 100 mg pure CBD by the oral and vaped routes were analyzed for Δ-9-THCCOOH, Δ-8-THCCOOH, 11-OHTHC, Δ-9-THC, and Δ-8-THC. There was no sign of conversion of CBD to Δ-9-THC or Δ-8-THC. There were traces (typically <2 ng/mL) of these analytes in some urine specimens, but these traces were detected similarly in all dosing conditions, including placebo. Blood specimens from the same participants were uniformly negative for the same analytes. Three of the 18 participants confirmed positive (≥15 ng/mL) for Δ-9-THCCOOH in urine following vaped administration of a single 100 mg dose of contaminated CBD. The concentrations, specimen collection times, immunoassay response, and creatinine are shown in the table for the positive specimens.

Conclusion/Discussion:

Although the conversion of CBD to Δ-9-THC and Δ-8-THC can occur in vitro, ingestion and vaping of pure CBD did not result in positive tests for Δ-9-THC or Δ-9-THCCOOH. There is the potential that CBD products that contain low concentrations of Δ-9-THC (analogous to those in legal hemp products) can produce positive drug tests for marijuana.

Driving Under the Influence Case Report Involving Flualprazolam and Marijuana

Authors and Affiliations: Charis Wynn*, Samantha Tolliver. Forensic Toxicology Division, Office of the Chief Medical Examiner, 401 E St. SW, Washington, DC 20024.

Background/Introduction:

Marijuana is the second most prevalent drug of abuse of the District of Columbia's Driving Under the Influence (DUI) population. Marijuana impairs cognition, psychomotor function, and driving performance. Similarly, flualprazolam, a fluorinated analog of alprazolam and a central nervous system depressant, also has adverse effects including sedation, reduced anxiety, impaired balance, incoordination, impaired cognitive abilities, confusion, and slurred speech. Many designer benzodiazepines cross-react with immunoassay screens yet may not confirm with a targeted benzodiazepine confirmation test. As a result; a novel approach for the analysis of designer benzodiazepines should be conducted. Additionally, referential mass spectral data may be necessary for identification and reporting of such drugs.

Objectives:

A case study is presented illustrating the effects of flualprazolam and marijuana on driving performance and standardized field sobriety testing (SFST). The presentation will also describe the urine flualprazolam and marijuana testing methodology utilized by the toxicology laboratory at the Washington, DC Office of the Chief Medical Examiner (DC OCME). Additionally, an evaluation of cross-reactivity of flualprazolam in enzyme linked immunosorbent assays (ELISA) will be presented.

Methods:

An 18-year-old female was suspected of driving under the influence of alcohol or drugs after colliding with a parked vehicle. The officer observed the individual to slur her speech, sway front to back while standing, and have a blank stare. She admitted to being on prescribed anxiety medication, alprazolam (Xanax), and smoking marijuana previously; therefore, field sobriety tests were administered. Driving under the influence cases submitted to the DC OCME were routinely screened by ELISA, liquid chromatography tandem mass spectrometry (LC-MS/MS) for synthetic cannabinoids in urine, and headspace gas chromatography flame ionization detection (GC-FID) for volatile compounds. The ELISA testing panel includes 12 (amphetamines, barbiturates, benzodiazepines, cocaine, phencyclidine, methamphetamine, methadone, opiates, oxycodone, marijuana, fentanyl, and buprenorphine) major drug classes. The ELISA benzodiazepines assay targets nordiazepam at a cutoff of 100 ng/mL and THCCOOH for marijuana at a cutoff of 50 ng/mL. Presumptive positive results in this case were confirmed by LC-MS/MS, gas chromatography coupled with mass spectrometry-nitrogen phosphorus detector (GC-MS-NPD), and liquid chromatography-quadrupole time of flight mass spectrometry (LC-QTOF-MS). The LC-MS/MS limit of detection for the marijuana metabolite, 11-nor-9-carboxy-THC, was 5ng/mL. Additionally, an ELISA cross-reactivity study was conducted with Orasure Technologies Benzodiazepines kit and a Cayman Chemical reference standard of flualprazolam to determine cross-reactivity.

Results:

On the SFST, the individual displayed a lack of smooth pursuit, distinct and sustained nystagmus at maximum deviation, and vertical nystagmus. While performing the Walk and Turn test, the individual broke her feet apart for balance several times as the instructions were given. She also raised her forearms about 90 degrees while walking, did not step heel-to-toe on several steps, stepped off the line while walking forward and back, had to be instructed to turn around to continue, moved both feet while turning, and counted 10 steps despite taking 9 steps. During the One Leg Stand, she swayed and put her foot down several times. A urine sample was then collected and submitted to the DC OCME for toxicological testing. The urine sample was presumptively positive for benzodiazepines and cannabinoids by ELISA. Subsequently, 11-nor-9-carboxy-THC was qualitatively confirmed by LC-MS/MS. Flualprazolam was detected by LC-QTOF-MS and had a library match by GC-MS-NPD. A 200 ng/mL sample of flualprazolam was 182.5% cross-reactive with the Orasure ELISA Benzodiazepine kit.

Conclusion/Discussion:

Officer observations correlated with the effects of flualprazolam as well as marijuana. Flualprazolam cross-reacts with Orasure's ELISA Benzodiazepine kit. Toxicologists should utilize analytical data available, such as full scan mass spectral library data, to detect flualprazolam if a selected ion monitoring method is not developed.

Impact of Legalization of Marijuana on Driving Under the Influence Cases in Washington, DC

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Background/Introduction:

In November 2014, the Legalization of Possession of Minimal Amounts of Marijuana for Personal Use Initiative (Initiative 71) was approved and became effective on February 26, 2015. Accordingly, marijuana is legally permitted on private property for a person who is at least 21 years of age. Under the law, it is permissible to possess two ounces of marijuana and transfer up to one ounce to a recipient that is at least 21 years of age. It is still illegal to sell, consume in public or operate a vehicle under the influence of marijuana.

Objectives:

It is vital to collect driving under the influence (DUI) data. Therefore, it was of interest to examine the impact of the legalization of marijuana on the DUI cases received at the toxicology laboratory in Washington, DC Office of the Chief Medical Examiner (DC OCME). As a result, this study will illustrate the demographics of DUI cases as it pertains to marijuana and evaluate the effect of the implementation of Initiative 71 on DUI cases in Washington, DC.

Methods:

A review of DUI cases (n=2,763) received from local and federal police agencies, in the Washington, DC area, from 2014-2019 was conducted. All DUI samples, blood or urine, received an immunoassay screen. The Limit of Detection (LOD) in blood by immunoassay was 25.0 ng/mL and the LOD in urine by immunoassay was 50.0 ng/mL. Blood specimens received from 2014-January 2016 were confirmed by gas chromatography mass spectrometry with a LOD of 2.5 ng/mL and Limit of Quantitation (LOQ) of 5.0 ng/mL. Beginning February 2016 the laboratory implemented an updated method by gas chromatography tandem mass spectrometry with a lowered LOD of 1.0 ng/mL and LOQ of 2.0 ng/mL. Urine specimens were confirmed at a LOD of 5.0 ng/mL by liquid chromatography tandem mass spectrometry. The majority (80.0%) of the marijuana-positive DUI cases were urine specimens; therefore, most cases were reported qualitatively. A data review of all reported DUI cases was then conducted on an internal data management system.

Results:

In 2014, 19.4% of the cases with marijuana present reported the subject's sex as female and 78.6% were reported as male. The remaining 2.0% did not report a sex with the laboratory submission form. Comparatively, in 2019, 23% of the cases with marijuana present reported the subject's sex as female and 68.5% were reported as male. The remaining 8.5% did not report a sex with the laboratory submission form. In 2014, the average age of all marijuana users was 32 years old ranging from 16-67 years of age. The average age for females and males was 33 and 31, respectively. Similarly, in 2019, the average ages of all marijuana users were 35 ranging from 17-72 years of age. The average age for females and males was 31 and 36, respectively. The prevalence of marijuana in driving under the influence cases increased from 22.8% in 2014 to 38.5% in 2019. Table 1 illustrates the total number of DUI cases received by the DC OCME and the number of marijuana detected DUI cases by year. The average number of DUI cases received is 460.5 per year.

Conclusion/Discussion:

In conclusion, since the implementation of Initiative 71, the prevalence of marijuana in DUI cases in the District of Columbia has nearly doubled while the amount of total DUI cases received has been consistent. Also, the demographics of DUI cases that detected marijuana since the implementation of Initiative 71 remained consistent predominately being males and most subjects were on average in their early to mid-thirties.

Mitragynine in Orange County DUID population

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Background/Introduction:

Mitragynine and 7-hydroxymitragynine are psychoactive components found in the drug marketed as Kratom. They are substances that have been on the market as a legal high and are commonly used as an opioid substitute or as an aid during detox. Mitragynine has μ-opioid receptor affinity and has shown dose dependent effects, acting like a stimulant at low doses and an opioid at higher doses. While the DEA was met with opposition trying to move the substance to a Schedule 1 drug, the California Vehicle Code defines a drug as "any substance that could so affect the nervous system, brain, or muscles that would cause impairment of your ability to drive as a reasonably cautious driver". Given that definition, information and observations gathered in a driving under the influence of drugs (DUID) investigation along with toxicology could be useful in determining if the effects of the drug render that individual impaired for the purposes of driving.

Objectives:

To look at the demographics, concentrations, concurrent drug use, and DUID performance of individuals with detectable levels of mitragynine in their blood at the time of driving.

Methods:

DUID samples over a 3 year period (January 2017- December 2019) were evaluated. All cases were screened by immunoassay for 7 drug classes, not including mitragynine, with the addition of liquid chromatography quadrapole time-of-flight (LC-QTOF) screening in August 2018 which did include both mitragynine and 7-hydroxymitragynine. Mitragynine was confirmed and quantitated using DPX WAX-S extraction with liquid chromatography with a tandem mass spectrometer (LCMSMS) analysis. Mitragynine was validated following SWGTOX guidelines for the calibration range of 10 – 640 ng/mL in blood. Police reports were collected for cases where mitragynine was reported and evaluated based on symptomology, driving performance, and field sobriety performance.

Results:

Between January 2017 and December 2019, there were 60 DUID cases positive for mitragynine and/or 7-hydroxymitragynine. The average mitragynine concentration was 109.2 ng/mL with a range of 10.5 ng/mL to >640 ng/mL. 7-hydroxymitragynine was qualitatively detected in 62.2% of the cases in which it was screened for. All of these cases had either alcohol or other drugs detected, with 36% having alcohol detected and an average of 2 other drugs classes besides alcohol detected. Cases including fentanyl represented 13% of the cases and cases with other opioids such as morphine, codeine, oxymorphone, oxycodone, hydrocodone, hydromorphone, and methadone representing 35% of the cases. Ninety percent of the population was male, 78% was Caucasian, and the average age was 32.3 years. The most common reason for contact was subject involvement in a traffic collision. An average of 4.4 cues was seen in the walk and turn performance, 2.5 on one leg stand performance, 4 misses on finger to nose, and 34.8 seconds estimate for 30 seconds on the Romberg with 68% demonstrating sway.

Conclusion/Discussion:

Mitragynine is present in the Orange County driving population, however its individual effects on driving and field sobriety test performance are not clear. Often taken in combination with other potentially impairing substances, it is likely contributing to impairment, but current data is unable to discern impairment caused solely by mitragynine. Due to the abuse of mitragynine with other drugs, association between blood concentrations and the stimulant or opioid-like effects cannot be made.

SOFTEMBER PLATFORM ABSTRACTS SESSION 5

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Blackout brownie: a final dessert case study

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Background/Introduction:

A 65-year-old female and her husband had previously tried gummy cannabis products as a compliment to other pain management treatments but had not been satisfied with the results. A request was made to their son to obtain some stronger edible cannabis product and he delivered a cannabis brownie, which according to the package contained 1000 mg of THC. After splitting the brownie between the two of them, both experienced extreme stomach pain and vomiting. After the husband went to shower, he noticed his wife had never come upstairs, and found her "stuck" on the fourth stair. In the morning, the husband came downstairs and found his wife deceased at the bottom of the stairs with no apparent trauma.

Objectives:

Toxicology results can play a critical role in determining cause of death (COD) and understanding the effects drugs have on the body can aid in determining both COD and manner of death. This case study is examined to explore possible factors that could have contributed to an individual's cause of death after ingestion of a cannabis brownie.

Methods:

Standard laboratory practices for postmortem analysis were followed in this case, including volatiles analysis via headspace gas chromatography mass spectrometry (GCMS), 3 panel drug screen by immunoassay, and liquid chromatography quadrapole time-of-flight (LC-QTOF) screening followed by appropriate confirmation analysis. Cannabinoids confirmation was performed via SLE extraction and liquid chromatography tandem mass spectrometry (LCMSMS). The method was validated following SWGTOX guidelines for the calibration range of 1 – 100 ng/mL for THC, Hydroxy-THC, CBD, and CBN and 5 – 500 ng/mL for Carboxy-THC in blood, urine, brain, and gastric contents. Validation for testing in liver is still underway and was performed for research purposes only in this case. All matrices were analyzed in duplicate to obtain quantitative results. Investigative notes taken from the scene as well as autopsy findings were examined to aid in understanding circumstances surrounding the death as well as pathological findings.

Results:

A full autopsy was performed in this case allowing for the testing of multiple samples. The results are listed in Table 1. The central postmortem blood also contained approximately 339 ng/mL pseudoephedrine and 250 ng/mL diphenhydramine. Solid dose testing of remnants of brownie collected from the wrapper did detect the presence of THC, however there was no procedure to quantitate that result. The autopsy report noted that the decedent had full blockage to one of the cardiac arteries with significant blockage to other arteries and the decedent was noted to be obese with a BMI of 38.6. Cause of death has been ruled as natural.

Conclusion/Discussion:

Today's cannabis culture has many individuals viewing the drug as a safe alternative or additive therapy to treat numerous ailments. Further, there are marked pharmacokinetic and pharmacodynamic differences seen when cannabis products are smoked compared to orally ingested. There does not yet seem to be a consensus or large enough body of research on cannabis and cardiotoxicity, but in a case such as this the question may remain for some time as to how much of a role an edible cannabis product had on an already cardiac compromised individual.

Cannabinoids Distribution in Postmortem Blood, Vitreous Humor, Urine, and Tissue Samples after Controlled Smoked Cannabis Administration to Rabbits

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Background/Introduction:

Cannabis is commonly abused worldwide for its euphoric effects. The plant contains over 100 different cannabinoids including Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), and tetrahydrocannabivarin (THCV). When smoked, the psychoactive component, THC, is quickly absorbed and distributed throughout the body, resulting in a rapid onset of action. Blood pharmacokinetics after smoked cannabis administration is well characterized in humans; however, there is limited research detailing the distribution of THC and other cannabinoids in tissues immediately after smoked cannabis administration.

Objectives:

To characterize cannabinoid distribution in postmortem samples obtained from rabbits following smoked cannabis administration.

Methods:

Adult female New Zealand white rabbits (n=3) were sedated, then administered cannabis smoke (6.8% THC) via a smoking machine and exposure chamber (Teague Enterprises, Woodland, CA) over a 40 min period. The rabbits were anesthetized, then euthanized by cervical dislocation 25 minutes after the last cigarette puff into the exposure chamber. Necropsy was performed immediately after death to obtain blood, urine, vitreous humor, bile, heart, lung, liver, spleen, kidney, muscle, and brain. THC, 11-nor-9-carboxy-THC (THCCOOH), 11-hydroxy-THC (11-OH-THC), THCCOOH-glucuronide, THC-glucuronide, CBD, CBG, CBN, and THCV were quantified in all biological specimens by a validated liquid chromatography-tandem mass spectrometry method. Linear dynamic ranges were 0.25 – 50 ng/mL for THC-g, 0.5 – 100 ng/mL for CBN, 1 – 100 ng/mL for 8β-diOH-THC, THCVCOOH, 8β-OH-THC, and THCV, 1 – 250 ng/mL for THCCOOH-g, and 0.5 – 250 ng/mL for 11-OH-THC, THCCOOH, CBD, CBG, and THC

Results:

THC, THCCOOH, and CBN were quantified in heart, cavity, and peripheral blood samples (Table 1). No other metabolites or cannabinoids were detected in blood. THC-glucuronide was the only cannabinoid detected in urine samples with concentrations of 8.9, 4.2, and 25.3 ng/mL for rabbits 1, 2, and 3, respectively. Mean (range) THC concentration (ng/g) in tissues was: lung, 487 (379 – 601); heart, 23 (12 – 42); kidney, 22 (10 – 40); brain, 21 (18 – 28); spleen, 18 (8 – 34); muscle, 13 (10 – 17); and liver, 9 (6 – 10). Liver and kidney were positive for THC-glucuronide, and CBN, CBG, CBD, and THCV were detected in lung. CBN was also detected in liver.

Conclusion/Discussion:

Controlled cannabis doses were administered to rabbits through an automated smoking device. This is the first study to report major and minor phytocannabinoid concentrations in various rabbit tissues after smoked administration of cannabis. Despite rapid necropsy and blood collection immediately postmortem, concentrations of THC in heart and cavity blood were higher than that in caudal abdominal blood in all rabbits, suggesting differential distribution of THC in the chest versus caudal body. In blood, liver, and kidney samples, a peak is detected that is similar to THCCOOH-glucuronide, but exhibits a retention time shift from the commercially purchased standard. Evidence supports the idea that the peak found in rabbit biological matrices is an isomer of the available THCCOOH-glucuronide form. Ramifications of these findings will be examined, as well as discussing this study's role in a larger, ongoing research project designed to evaluate the postmortem redistribution of cannabinoids in rabbits

Cases Analyzed for Chloroethane Over an 11-year Period

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Background/Introduction:

Chloroethane is a colorless gas that has a distinguishable odor. It is also referred to as ethyl chloride. Chloroethane is used commercially in industry and in pharmaceuticals. Recreationally, chloroethane is an inhalant pursued by users for its narcotic effects. It is commonly sold under the name “Maximum Impact”. Inhalation of high concentrations of chloroethane vapor can be lethal.

Objectives:

This study sought to examine the blood concentrations associated with chloroethane use and to determine the demographics of users including age, gender, and location based on the case information provided.

Methods:

For this study, analytical data and accompanying case information were reviewed from 107 blood samples testing positive for chloroethane from January 2009 through March 2020. Chloroethane was analyzed using a dual column headspace gas chromatography with flame ionized detection (GC/FID).

Results:

One hundred seven (107) samples were analyzed that included both antemortem and postmortem cases. Seventy-six (76) were quantitatively measured with a mean concentration of 28 ± 27 µg/mL, median of 18, and range of 2.0 µg/mL to 120 µg/mL. The limit of quantitation was 2.0 µg/mL. The remaining 31 samples were reported qualitatively. The samples were reported qualitatively when the concentration of the result exceeded the highest calibrator or when two measured concentrations were outside the acceptance limits for quantitative agreement likely due to the volatility of the substance. When provided, the age of the individuals in these cases ranged from 25 to 74 years, with a mean of 46 ± 9.9 years and a median of 47 years. The gender identified in 104 of these cases was male with the 3 remaining cases unspecified. There was a representation from 30 of the 50 states in the United States and Puerto Rico. Other drugs were detected in 64 of the 107 cases through the drug testing of the samples submitted. Ethanol, caffeine, and marijuana were the most common drugs detected in combination with the presence of chloroethane. Of the 43 remaining cases, 37 cases only requested testing for volatile organic compounds. A case history was provided in 29 of the 107 cases reviewed, most of which were indicative of suspected inhalant abuse.

Conclusion/Discussion:

Overall, blood concentrations were broadly distributed over the samples analyzed. Chloroethane has been abused over the years because of its desirable narcotic effects. According to the data collected from samples tested, chloroethane was detected predominantly in middle aged males throughout the U.S. Testing for volatile organic compounds can be an important component of a comprehensive drug screen in detecting and identifying substances of abuse.

Positivity Rates in Infant Drug Exposure through Umbilical Cord and Postmortem Blood Testing

Authors and Affiliations: Jennifer L. Turri Swatek*, Timothy M. Yannaccone, Laura M. Labay NMS Labs, Horsham, PA

Background/Introduction:

Neonatal Abstinence Syndrome (NAS) manifests as a group of problems that occurs in infants going through drug withdrawal shortly after birth. Symptoms include seizures, poor feeding, excessive crying, and rapid breathing. Umbilical cord tissue provides an immediate, non-invasive specimen source that can undergo rapid testing for diagnostic insight. In addition to clinical diagnostic purposes, toxicology testing is included as part of a Sudden Unexpected Infant Death (SUID) investigation to determine the role that drug exposure, if any, may have contributed to death. Depending upon the age at the time of death, positive findings may be representative of in utero and/or other exposure routes.

Objectives:

This presentation provides insight into pediatric exposure to drugs of abuse by comparing umbilical cord results with infant (1 month - 1 year old) postmortem populations.

Methods:

Analytical data collected over a 1-year period (July 2019 – June 2020) from umbilical cord and postmortem casework in a population were compared. Umbilical cord specimens underwent screen and confirmation testing via High Performance Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS). Postmortem blood cases were screened by High Performance Liquid Chromatography/Time of Flight-Mass Spectrometry (LC/TOF-MS) and confirmation testing was performed by LC-MS/MS or Gas Chromatography/Mass Spectrometry (GC/MS). Only drugs covered in both analytical scopes were evaluated.

Results:

A total of 5575 positive umbilical cord and 240 postmortem cases had positive findings that were also included in the umbilical cord test panel. Postmortem data was divided into the age groups less than one month old (n = 87 cases) and 1 month old to 1 year old (n = 153). To eliminate the uncertainty of possible exposure occurring while in utero, the less than one month old age group was not included in the data.

Conclusion/Discussion:

Of a total 13794 umbilical cord cases submitted for analysis, the positivity rate was about 40%. Of a total of 1906 neonate and infant cases submitted for analysis, the positivity rate was about 12% for analytes covered in the same scope as umbilical cord testing. A consideration for the variation of positivity rates between these two populations would be the clientele that submits casework; umbilical cord may only be submitted for testing in cases where drug exposure is suspected while blood is submitted during routine postmortem examinations. Ethanol was identified in both populations; however, the possibility of postmortem formation needs to be considered. In addition, medical administration of some drugs account for an unknown number of the positive cases in both populations. Overall, the presented data provides insight into the drugs that an infant, aged 1 month to 1 year, may be exposed to in utero or their physical environment(s).

Unintended Consequences of Vaping: Impacts on Human Performance and Postmortem Casework

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Background/Introduction:

The Armed Forces Medical Examiner System Division of Forensic Toxicology has experienced a dramatic increase in synthetic cannabinoid cases since early 2019. This trend has coincided with the popularization of cannabidiol (CBD) products and e-cigarette use. While CBD is a non-intoxicating phytocannabinoid, some CBD-containing products may be adulterated with synthetic cannabinoids, over-the-counter medications, or other drugs of abuse. Acute effects of synthetic cannabinoids include cognitive and psychomotor impairment, dilated pupils, reddened conjunctiva, nausea and vomiting, hypertension, tachycardia, and sweating. More serious toxicity may manifest as myocardial infarction, stroke, or seizures. Some e-cigarettes allow the user to modify intake of e-liquids, which may lead to larger doses of these potent substances.

Objectives:

Describe pharmacological effects observed in 95 human performance cases and scene findings from a postmortem case involving vaporized synthetic cannabinoid exposure.

Methods:

Urine and blood specimens were submitted to the Division of Forensic Toxicology for analysis. Testing was assigned depending on case type (e.g., general investigation, driving while intoxicated, drug facilitated sexual assault, postmortem) and case history. Synthetic cannabinoids and metabolites were screened and confirmed by LC-MS/MS with an LOD of 0.1 ng/mL, except 5F-PB-22 3-carboxyindole (LOD 0.2 ng/mL). Analytes were qualitatively reported.

Results:

Between January 2019 and August 2020, the Division of Forensic Toxicology confirmed 342 synthetic cannabinoid cases. Of these, 28% listed vaping in the case history. Several synthetic cannabinoids were detected in cases with a history of vaporized “CBD oil”, including metabolites of 5F-ADB, 5F-AMB, FUB-AMB, 4F-MDMB-BUTINACA, MMB-FUBICA, 5F-MDMB-PICA, and 5F-PB-22. In January of 2020, the first vaping-related case involving MDMB-4en-PINACA 3,3-dimethylbutanoic acid was reported. Since then, 30 additional vaping-related cases have included this synthetic cannabinoid metabolite, either alone or in combination with other synthetic cannabinoids or metabolites. In cases where additional substances were analyzed, 11-nor-9-carboxy-Δ9-tetrahydrocannabinol was identified most frequently. The most common acute intoxication effects observed in 342 synthetic cannabinoid cases included slurred or incoherent speech, sluggish or groggy behavior, impaired motor skills and balance issues, disorientation, slow and delayed responses, and glassy or watery eyes. Several confirmed positive synthetic cannabinoid vaping cases will be highlighted, including a 22 year-old male driver who crossed into oncoming traffic and struck an electrical pole. The vehicle rolled and was found upside down. A clear liquid substance was found in a vape pen inside the vehicle. In a second case, a 20 year-old male jumped out of a moving vehicle, leaving the passenger of the vehicle to assume control. A vaping device was located in the driver’s pocket when he was apprehended. Also, a postmortem case will be highlighted in which a 25 year-old male was found deceased under his bed. Investigators found 2 vaping devices and multiple empty vaping liquid containers at the scene.

Conclusion/Discussion:

Although often perceived as innocuous, vaping devices found at the scene of an accident or death may provide valuable clues to direct appropriate forensic toxicology testing. E-cigarette products may contain substances other than nicotine, which have the potential to cause intoxication. Individuals using these products may be unable to safely operate a motor vehicle, or may experience serious adverse events, leading to death. Synthetic cannabinoid intoxication may appear similar to other drugs of abuse, resulting in negative toxicology results through traditional approaches. It is recommended

that additional testing for modern synthetic cannabinoids be performed in similar intoxication cases when other drugs of abuse are not found.

SOFTEMBER PLATFORM ABSTRACTS SESSION 6

Society of Forensic Toxicologists Inc.®

Casework is still Spice-y

Authors and Affiliations: Jeffrey P. Walterscheid*, Jami D. Reber, Ryanne T. Brown, Hao-Jung Chun, John J. Kristofic, Jessica L. Knittel, Grace A. Connolly, and Erin L. Karschner Division of Forensic Toxicology, Armed Forces Medical Examiner System, 115 Purple Heart Dr., Dover AFB, DE 19902

Background/Introduction:

The recent rise of synthetic cannabinoid use has correlated with increased availability of concentrated liquids or "juice" formulated for vaping by electronic cigarettes. Most e-liquids are sold as flavored nicotine solutions; however, others posing as CBD oil may contain new synthetic cannabinoid variants. These compounds change regularly as the DEA adds new substances to the Controlled Substances Act. When new compounds arise, targeted screening methods may fail to identify a substance in a suspected intoxication case. In these situations, it is crucial to investigate extraneous peaks appearing in retention time windows and to use exact mass data and chemical formulas to elucidate novel analytes appearing in casework.

Objectives:

This presentation raises awareness on how to identify and confirm prominent synthetic cannabinoid derivatives emerging and circulating in current forensic casework.

Methods:

Blood and/or urine specimens were submitted to our laboratory as part of suspected driving while intoxicated or military fit-for-duty investigations. Routine screening encompassed a drugs of abuse immunoassay for 9 drug classes and an alcohol screen by headspace gas chromatography. If case history indicated potential CBD use, a phytocannabinoid panel and a validated synthetic cannabinoid LC-MS/MS screen was utilized. If the LC-MS/MS synthetic cannabinoid screen was negative and case history indicated an intoxicating substance, an analysis was conducted via liquid chromatography time of flight mass spectrometry to gather additional information. Presumptively positive screens were confirmed by LC-MS/MS analysis with a limit of detection at 0.1 ng/mL.

Results:

A retrospective look at cases from April 2019 to present day revealed the rise and persistence of 4F-MDMB-BUTINACA and 5F-MDMB-PICA as the most prevalent species found, particularly as metabolites in both blood and urine. In all, 185 cases involving one or more synthetic cannabinoid(s) were characterized during 2019. Importantly, several blood cases would have been reported negative if only parent compounds were pursued, highlighting the need to analyze metabolites in blood. By 2020, a new compound known as MDMB-4en-PINACA emerged, where the typical indazole chain terminates with an unsaturated alkene instead of a saturated alkyl group or fluorine. This new variant became one of the most common findings either alone or in combination with 4F-MDMB-BUTINACA, 5F-MDMB-PICA, and/or their metabolites in half of all new cases. Many of these cases involving MDMB-4en-PINACA came from several states across the country, but the bulk of positives originated from outside the United States. Interestingly, 5F-PB-22 3-carboxyindole directly correlated with the presence of 5F-MDMB-PICA, raising the possibility of a convergent metabolic pathway.

Conclusion/Discussion:

With the military's zero-tolerance for cannabis use, some service members have turned to products containing synthetic cannabinoids. Modern formulations may contain an ever-changing selection of synthetic cannabinoids that pose a threat to personnel health, safety, and mission readiness. These results demonstrate the importance of staying abreast of current trends, frequently updating methods with relevant synthetic cannabinoid compounds, and conducting synthetic cannabinoid analyses as part of routine forensic toxicology casework.

Development and Validation of an LC–MS/MS Method for the Detection of 40 Benzodiazepines and Three Z-Drugs in Blood and Urine by Solid-Phase Extraction

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Background/Introduction:

Benzodiazepine (BZD) pharmacotherapies are prescribed for the treatment of anxiety, depression, sleep disorders, seizures and presurgical anesthesia induction. A 2012 retrospective study of heroin deaths in Cuyahoga County examined decedents' prescription records from the Ohio Automated Rx Reporting System. After prescription opioids, BZD were identified as the second most abundant class of compounds prescribed to decedents. Due to the BZD sedative effects, their detection is common in driving under the influence of drugs (DUID) and drug-facilitated sexual assault (DFSA) cases. Further, novel BZD were introduced in recent years and are not controlled under international drug control laws. The majority of BZD methods published to date were developed for either blood or urine, with only a few for both matrices.

Objectives:

Develop a comprehensive liquid chromatography – tandem mass spectrometry (LC–MS/MS) method for the analysis of (±)-zopiclone, 2-hydroxyethylflurazepam, 3-hydroxyflunitrazepam, 4-hydroxyalprazolam, 7-aminoclonazepam, 7-aminoflunitrazepam, alprazolam, bromazolam, clobazam, clonazepam, clonazolam, delorazepam, deschloroetizolam, diazepam, diclazepam, estazolam, etizolam, flualprazolam, flubromazepam, flubromazolam, flunitrazepam, flunitrazolam, flurazepam, lorazepam, lormetazepam, meclonazepam, methylclonazepam, midazolam, N-desalkylflurazepam, N-desmethylclobazam, N-desmethylflunitrazepam, nitrazepam, nitrazolam, nordiazepam, oxazepam, phenazepam, temazepam, triazolam, zaleplon, zolpidem, α-hydroxyalprazolam, α-hydroxymidazolam and α-hydroxytriazolam in 0.5 mL whole blood and urine in order to investigate postmortem, DFSA and DUID cases.

Methods:

Urine samples were hydrolyzed at 55ºC for 30 min with a genetically-modified β-glucuronidase (IMCSzyme®) enzyme from Micro-Chromatography Systems (Columbia, SC). Blood and urine samples underwent osmotic lysing, precipitation with 1.5 mL ice-cold 10:90 methanol:acetonitrile solution, freezing for 10 min and centrifugation for 10 min at 2,800 x g. Extraction was accomplished by SPE using Strata® Screen-C, 200 mg/6mL extraction cartridge from Phenomenex (Torrance, CA). Analysis was performed by Thermo Scientific™ Vanquish™ Flex LC system coupled with TSQ Quantis™ MS/MS (Thermo Scientific™, San Jose, CA). Chromatographic separation was accomplished with a solid-core Accucore™ Phenyl-Hexyl column (2.6 µm, 150 x 2.1 mm, 80Å, 130 m2/g; Thermo Scientific™). The mobile phases were 0.1% formic acid in LC-MS grade water and 0.1% formic acid in acetonitrile. The flow rate was 0.5 mL/min. The column oven and autosampler were maintained at 40 and 5°C, respectively. The spray voltage was set to 3.5 kV. The vaporizer and ion transfer tube temperatures were 350 and 250°C, respectively. Sheath and auxiliary nitrogen gas pressures were 50 (~5.58 L/min) and 10 (~7.97 L/min), respectively. Argon at 1.5 mTorr was the collision gas. Chromeleon™ 7.2 SR4 and Thermo Xcalibur™ 4.1 software were utilized to operate the LC. TSQ Quantis™ 3.0 SP1 and TraceFinder™ 4.1 SP3 software packages were used for data acquisition and analysis.

Results:

Twenty-nine of 43 analytes were quantified in 0.5 mL whole blood and qualitatively identified in urine. The four dynamic ranges of the seven-point, linear, 1/x weighted calibration curves with lower limits of quantification of 2, 5, 10 and 20 µg/L across the analytes encompassed the majority of our casework encountered in postmortem, DFSA and DUID samples. Bias and between-and within-day precision for QCs were all within ±15%, except for clonazolam and etizolam that were within ±20%. Twenty-five analytes met all quantitative reporting criteria including dilution integrity, and 14 analytes met qualitative reporting criteria in both matrices. Greater than 95% hydrolysis efficiency was achieved as measured with

oxazepam glucuronide. Urine matrix was qualitatively validated against the blood calibration curve.

Conclusion/Discussion:

This method features exceptionally high signal-to-noise ratios and provides a comprehensive prescription and designer BZD panel. The ability to analyze quantitative blood and qualitative urine samples in the same batch is one of the most useful elements of this procedure. This sensitive, specific and robust analytical method was routinely employed in the analysis of greater than 300 samples in our laboratory over the last six months.

Development of an Untargeted Gas Chromatography-Mass Spectrometry (GC/MS) Method for the Detection of Drugs in Wastewater

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Background/Introduction:

Monitoring current illicit drug trends and consumption rates of pharmaceuticals using a non-invasive collection technique is needed to address the present drug use and the growing drug epidemic. Reliance on self-reporting drug use surveys is not always a practical measure of illicit drug use. Wastewater analysis has been used globally as a targeted method for monitoring the consumption of specific illicit drugs. Current existing analytical techniques for wastewater analysis focus on the use of targeted liquid chromatography-mass spectrometry (LC-MS) based techniques. Few gas chromatography (GC) procedures exist for wastewater analysis, and those that do concentrate their methods on a single class of drugs operating their mass spectrometer (MS) in selective ion monitoring (SIM) mode.

Objectives:

The purpose of this study was to develop an untargeted, underivatized, full scan gas chromatography-mass spectrometry (GC/MS) method for the analysis of wastewater.

Methods:

Solid phase extraction (SPE) was performed with UCT mixed mode, 15 mL Clean Screen DAU columns with 500 mg sorbent to extract a 500 mL wastewater sample. Sample extracts were reconstituted in ethyl acetate and analyzed on a Shimadzu GCMS-QP2020 gas chromatograph-mass spectrometer (GC/MS) installed with an Agilent J&W HP-5MS (30 m × 0.25mm, 0.25 µm) column. Injection volume, flow rate, oven temperature, and ion source scan rate were optimized to develop an untargeted full scan method for the detection of pharmaceuticals. Calibration curves were developed for 42 targeted drugs.

Results:

A 1 µL sample volume is run with a splitless injection utilizing helium as the carrier gas and the instrument operated in constant flow mode at a rate of 1.0 mL/min. The GC oven program is held at the initial temperature of 70°C for 2 min then ramped at 15°C/min to 300°C and held for 10 min for a total run time of 27.33 min. The injection port, transfer line, and source were set at 250°C, 280°C, and 200°C respectively. The MS operated in full scan mode, scanning ions from 35-550 m/z at an event time of 0.20 seconds from 3.50-27.33 minutes. Out of a 42 drug panel, over 75% of the generated calibration curves were suitable for quantitation with coefficients of determination greater than 0.9875. Limits of detection for most drugs ranged from 0.10-1.0 ng/mL, on par with many targeted liquid chromatography methods.

Conclusion/Discussion:

The optimized untargeted method is able to detect a wide range of compounds in addition to those in the drug panel. The untargeted full scan MS method supports monitoring a wider range of pharmaceuticals overlooked in traditional targeted waste water methods such as changing trends in novel psychoactive substances. This project was supported by the National Institute of Health (NIH), Award P30DA033934.

Isotonitazene: A Novel Benzimidazole μ-Opioid Agonist as the Latest Novel Opioid

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Background/Introduction:

Opioids remain the most frequently detected drugs in postmortem toxicology casework, with fentanyl being the most frequently detected across the United States. After the disappearance of most fentanyl analogs in late 2019 following core structure scheduling, novel opioids began appearing in forensic toxicology casework. Isotonitazene, a benzimidazole μ-opioid agonist discovered in 1957, first appeared in apparent opioid related deaths in the United States as early as April 2019, and as of April 2020 was the most prevalent novel opioid (besides carfentanil) in our laboratory's death investigation casework. Isotonitazene has a potency in animal models equivalent to that of fentanyl and is therefore a significant finding in death investigation casework.

Objectives:

We report the identification of isotonitazene in postmortem toxicology casework, including analytical characteristics, concentrations in death investigation cases, patterns of combined drug use and circumstances, and cause and manner of death in a series of up to 61 cases. In addition, we report the spread of the drug throughout the mid-western United States, from initial cases centered around Illinois, with evidence of widespread use in eastern and southern states by spring of 2020.

Methods:

Toxicology cases were identified as presumptively positive for isotonitazene based on data-mining results from liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS, Agilent Technologies, Santa Clara, CA) data files. Samples were acquired during routine screening procedures of postmortem blood samples at NMS Labs (Horsham, PA). Quantitative confirmation of isotonitazene was performed at CFSRE (Willow Grove, PA) and NMS Labs using standard addition, employing a blank and three up-spikes. At CFSRE, confirmation was performed using a Xevo TQ-S micro LC-MS/MS (Waters Corporation, Milford, MA). Chromatographic separation was achieved using gradient elution of 0.1% formic acid in water and 0.1% formic acid in methanol over an Agilent InfinityLab Poroshell 120 EC-C18 (3.0 x 100 mm, 2.7 μm) analytical column. The flow rate was 0.4 mL/min. Injection volume was 5 μL. Column temperature was 30°C. Following ESI+, multiple reaction monitoring (MRM) was used for mass detection. Based on recommendations by the Organization of Scientific Area Committees for Forensic Science (OSAC) for forensic toxicology, three transitions were monitored for isotonitazene to increase specificity, since the two most abundant fragment ions were derived from the same portion of the structure.

Results:

During the reporting period (mid 2019 to early 2020), isotonitazene was presumptively identified between NMS Labs and CFSRE in more than 150 cases, however, quantitative confirmatory testing was authorized in only 61 cases. The average concentration in blood was 2.2 ± 3.1 ng/mL (median 1.1 ng/mL; range 0.23–17 ng/mL). Isotonitazene was most frequently found in combination with designer benzodiazepines, including flualprazolam (69%) and etizolam, and less commonly found with other illicit opioids, including fentanyl, heroin, tramadol and U-47700. In approximately half the cases, isotonitazene was the only opioid present. For cases in which isotonitazene was present, the manner of death was typically ruled accidental and the cause of death was typically categorized as single or multiple drug intoxication involving fentanyl, etizolam and/or flualprazolam. Initial cases in this series originated from Illinois, Indiana and Wisconsin in 2019, and subsequently spread to neighboring states and Tennessee, South Carolina and Florida by early 2020. The earliest case to date occurred in April 2019 from a medical examiners office in Iowa.

Conclusion/Discussion:

During this time period, isotonitazene was the only benzimidazole drug detected from this series. It is hypothesized that

other related drugs including etonitazene, metonitazene, and clonitazene or their analogs may emerge in the future. Combination of isotonitazene with flualprazolam or etizolam was common suggesting that these designer drugs are packaged and distributed in combination. The pattern of spread suggests that the drugs are distributed through a network as opposed to random online internet purchases. The potency of isotonitazene will require detection limits achievable only by LCMSMS or LCTOF/LCQTOF screening methods.

Keeping your guard up in the context of validated methods: the example of a time-evolving bias created by the spiked matrix

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Background/Introduction:

A method rolled out in production following validation should still be monitored for potential issues and improved as necessary. Rather, monitoring the behaviour of various method's indicators should be weaved in daily operations to catch early indications of quality issues. This monitoring is of paramount importance in the case of court related toxicological analysis.

Objectives:

One instance of a quality issue emerging while the method was in production is presented here. A serious quantification bias problem was caught via subtle signs. The investigation performed is detailed, together with the corrective actions implemented.

Methods:

This issue occurred in a wide-scope targeted method quantifying 85 analytes in blood and screening for 51 additional compounds. Seven calibration standards and three quality controls (QCs) were spiked in whole blood purchased from UTAK (USA). This blood was supplemented with the antiglycolytic agent sodium fluoride and the anticoagulant potassium oxalate. The same preservatives are used in casework (grey-top BD Vacutainer tubes). Samples were extracted by protein precipitation and analysed by LC-MS/MS, with four different chromatographic methods: generic, cannabinoids, high therapeutic concentration compounds, novel psychoactive substances.

Results:

The first noticed symptom was a >50% gap in peak area of the internal standard THC-D3 between blood case samples and spiked samples (standards and QCs) which suggested a lower recovery in spiked blood. Nonetheless, all QC accuracies were found to be within tolerance limits (±25% for low level, ±20% for medium and high levels). Investigation to understand the source of this behaviour led to the blood matrix used to spike standards and QCs. Blood lots displaying this "gap effect" also result in a biased quantification in case work blood matrices (up to 296% for low concentrations). This was especially worrying in the context of a per se limit for THC at a low concentration. The bias evolves over time for a given blood lot, with recovery dropping from 97% to 41% over a 3 months period. The primary analyte impacted by this issue was Δ9-tetrahydrocannabinol, but effects were also noted for bupropion, fluoxetine, nortriptyline, olanzapine, paroxetine, sertraline and 11-hydroxy-Δ9-tetrahydrocannabinol.

Conclusion/Discussion:

Post-column infusion tests showed that this could not be linked to an ionization suppression effect. It would thus appear that blood ageing generates compound(s) interfering with the recovery or short-term stability of several analytes. If this is a recovery issue, one might wonder why internal standards do not compensate for this phenomenon. Results show it has very quick kinetics. Standards and QC are first spiked with the targeted analytes, and internal standards are then added as the batch is extracted. This short delay means internal standards and analytes do not undergo the process to the same exact extent, which is sufficient to prevent adequate tracking by internal standards. The issue was solved by switching to on site collection of blood from personnel, which allowed a better control over frequency of collection (and thus age of the blood matrices used for spiking), collection containers and cold chain. This collection process is on a voluntary basis and remains completely confidential for the donors. Furthermore, additional controls were implemented to monitor whether recovery was decreasing for blood lots used to spike standards and QCs. Further investigation will

be required to identify the precise mechanisms behind the creation of such a bias, which has important implications for results reported by toxicology laboratories.

SOFTEMBER PLATFORM ABSTRACTS SESSION 7

Society of Forensic Toxicologists Inc.®

Assessment of urinary buprenorphine, norbuprenorphine and naloxone concentrations in patients in prescription drug monitoring programs

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Background/Introduction:

Buprenorphine (BUP) with or without naloxone (NAL) is used for the treatment of opioid-dependent individuals. BUP is metabolized by N-dealkylation to active norbuprenorphine (NBUP). Measurement of these compounds is useful to assist in the clinical management of patients in a treatment program.

Objectives:

This retrospective study was performed to evaluate BUP/NAL administration in a population in the Southwest, United States. The objective of the study was to evaluate the parameters that reflect adherence to treatment.

Methods:

We analyzed deidentified results from consecutive random urine specimens submitted for BUP screening by immunoassay (CEDIA®, AU5800 analyzer) between July-October 2019. A cutoff concentration of 5 ng/mL was utilized with quality control (QC) material at 0, 3 and 7 ng/mL. Presumptive positive specimens were spiked with deuterated internal standards and prepared by solid phase extraction with SPEware/Tecan ALD II automated liquid dispenser into 96 well plates. Samples were analyzed for total BUP, NAL, and NBUP by liquid chromatography tandem mass spectrometry (LC-MS/MS) using a Prelude LX-4MD LC (Thermo) and SCIEX 4500 with a Kinetex Biphenyl, 50 x 3.0mm, 2.6 mcm, 100A column (Phenomenex). Eight calibrators (0.8-2000 ng/mL, Cerilliant) produced a quadratic curve fit with 1/x weighting. QC included drug free urine and 2.5, 800 (hydrolysis) and 1600 ng/mL. The cut-off was 2 ng/mL. Analyte resolution was achieved with a gradient binary mobile phase consisting of an aqueous phase of water and 0.1% formic acid, and an organic phase of methanol and 0.1% formic acid. Creatinine (CR) was determined by a modified Jaffe procedure with a reporting range of 1-350 mg/dL.

Results:

Of 8,970 specimens screened for BUP by immunoassay, 2,388 (26.6%) were presumptively positive. False positives (N=195, 8.2%), those which could not be tested, or the CR was outside the normal range or the BUP, or NBUP concentrations were >2000 ng/mL were excluded. This resulted in 1,840 specimens including 809 with NAL between 2-2,000 ng/mL. For those specimens screened for BUP the mean and median CR was 131.8 and 118.9 mg/dL, respectively. For those specimens for which BUP, NBUP were identified by LC-MS/MS (N=2,171), mean and median CR levels were 141.4 and 131.9 mg/dL, respectively. In comparison, the mean and median CR for all urine samples within the study period, were 128.8 and 114.9 mg/dL, respectively. Twelve specimens (<1%) were negative for BUP and positive for NBUP and 36 (<2%) were negative for NBUP and positive for BUP. BUP and NBUP concentrations <500 ng/mL comprised 92.2% and 62.9% of the samples, respectively. The Table shows the concentration data including correcting for CR (ng/mg CR). The regression equation of [BUP] vs [CR] was [BUP]=0.901[CR] + 61.4 with an r2=7.9%. The metabolic ratio [NBUP/BUP] calculated using CR normalized concentrations demonstrated a mean ±SEM of 3.9±0.084 and a median of 3.07. The regression equation of [NBUP] vs [BUP] was [NBUP]=0.954[BUP] + 300.5 with r2=25.9%. Most of the variation (%CV = 92.3%) was observed in samples with a [BUP] <100 ng/mL.

Conclusion/Discussion:

We evaluated the utility of measuring concentrations of BUP and NBUP for assessing adherence to addiction treatment. We also assessed the impact of correcting for renal clearance using CR. Poor correlation between BUP and CR was observed. We found that CR-corrected NBUP mirrored NAL concentrations and were nearly 4-fold higher than BUP, although they were poorly correlated, especially below 100 ng/mL. Further studies will assess the utility of the CR-corrected NBUP/BUP ratio for evaluating patient adherence to an addiction management program.

Evaluation of alcohol markers in urine and oral fluid after Kombucha consumption

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Background/Introduction:

Kombucha is a fermented beverage made from tea, sugar, yeast, and bacteria. The fermentation naturally results in low amounts of ethanol. According to U.S. federal regulations, beverages can be labeled as "non-alcoholic" if <0.5% alcohol content by volume (ABV). However, studies have demonstrated that some Kombucha drinks labeled as non-alcoholic actually contained 1.25–2.03% ABV. This raises particular forensic concerns because: 1) unintentional intoxication may result after consumption of these "non-alcoholic" drinks, or 2) individuals may use the consumption of Kombucha as an excuse for intoxication or impairment in workplace drug testing or driving while intoxicated cases. Despite the increasing popularity of Kombucha, little to no data exist which examine the impact of Kombucha consumption on testing for alcohol markers in biological specimens.

Objectives:

The objective of the study is to assess the presence of alcohol markers, ethyl glucuronide (EtG) and ethyl sulfate (EtS), in oral fluid and urine collected after controlled administration of a single Kombucha beverage in an IRB approved study.

Methods:
Twelve study participants provided written consent (n=6 males, n=6 non-pregnant females). Participants provided urine voids (V0) and Oral-EzeTM collections of oral fluid specimens (t0) before the consumption of one Kombucha (16 oz., claiming <0.5% ABV) within 20 min. Participants received one of two flavors (n=6 blueberry ginger, n=6 raspberry lemon ginger). Oral fluid specimens were collected ten minutes after the entire drink was consumed (t0.5h) and at 1, 3, 5, 8, 10, 24, 32, and 48 h relative to the start of drinking. Participants provided all urine voids for the first 12 h and then the first urine void on days 2 and 3. Participants were asked to abstain from all alcohol consumption during the study. Oral fluid specimens were screened for EtS (25 ng/mL cutoff) by liquid chromatography tandem mass spectrometry (LC-MS/MS). All urine specimens were analyzed by immunoassay (IA) (500 ng/mL EtG cutoff) and LC-MS/MS for EtG (500 ng/mL cutoff) and EtS (100 ng/mL cutoff). Quantitative values were not normalized for creatinine. Kombucha beverages were also evaluated by headspace gas chromatography to determine ABV. Specimens were considered positive if EtS was present with or without EtG above these limits; EtG-only samples were not considered positive.

Results:

Kombucha beverages contained 0.6–1.0% ABV and alcohol content was significantly higher in blueberry ginger samples compared to raspberry lemon ginger. All oral fluid specimens were negative for EtS. In urine, EtG and EtS were detected by LC-MS/MS together in 41.7%, 83.3%, 50.0%, 41.7%, and 8.3% of participants for the 1st through 5th urine voids. Two participants never had detectable EtG and/or EtS above the LC-MS/MS cutoffs. Urine voids on days 2 and 3 did not contain alcohol markers above the IA or LC-MS/MS cutoffs. The mean (range) urine EtS concentrations were 462 (100–3700) ng/mL while EtG were 1010 (530–2200) ng/mL. For EtG >500 ng/mL specimens, there was one false negative result and ten true positive results when comparing IA to LC-MS/MS. Interestingly, 16 urine specimens were positive for only EtS (compared to 10 samples positive for both EtG and EtS); these specimens screened negative by IA. Among these 16 EtS-only results, there was an EtG chromatographic interference observed in 8 specimens. Reanalysis on dilution was attempted but a quantitative EtG result could not be provided on these samples.
Conclusion/Discussion:
Consumption of Kombucha could potentially yield positive urine results for alcohol markers following consumption of a single beverage. Both Kombucha flavors contained more alcohol than allowable or advertised on the bottle. If labs were to only rely on immunoassay, they could potentially miss EtS-positive specimens. With regards to compliance monitoring or workplace drug testing, individuals may test positive in urine but not oral fluid for alcohol markers following consumption of "non-alcoholic" Kombucha.

Evaluation of Δ8- and Δ9-tetrahydrocannabinol co-elution in blood samples

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Background/Introduction:

In June of 2018 an intermittent interfering compound was recognized in the analysis of Δ9-tetrahydrocannabinol (Δ9-THC) in blood specimens. As a result, certain cannabinoid cases were indeterminate for Δ9-THC analysis. However, the interferent was not observed again for some time, so identification was not immediately pursued. In late 2019 as the interferent reappeared, seized drugs analysis identified Δ8-tetrahydrocannabinol (Δ8-THC) in a vape pen and subsequently a reference standard was obtained. Δ8-THC is an analogue of Δ9-THC with similar pharmacologic properties, but with lower psychotropic potency. It is naturally occurring in the marijuana plant in very low concentrations but can also be produced by conversion from Δ9-THC. The Δ8-THC standard was diluted in blood and extracted per the Δ9-THC method to confirm it would be detected based on ion transitions, ion ratios (IR) and retention time. With a positive finding, the next step was to determine if Δ9-THC could continue to be quantified in the presence of Δ8-THC or if findings would be qualitative until a separation method could be validated.

Objectives:

The objective of this research was to determine if Δ9-THC could be reliably detected and quantitated in the presence of co-eluting Δ8-THC.

Methods:

Blank porcine blood was fortified at varying concentrations with Δ9-THC and Δ8-THC. Δ9/Δ8 ratios ranged from 0.1-10. Each prepared blood was sampled at 150 mcL. Formic acid buffer (0.1%) and deuterated Δ9-THC internal standard were added as part of the automated extraction. This mixture was applied to a Biotage ISOLUTE SLE+ plate and eluted with 30:70 hexane:ethyl acetate. Samples were then dried under heated airflow and reconstituted in 40:60 ultra pure water:acetonitrile mobile phase. Extracts were analyzed by LCMSMS on a Waters Aquity UPLC using an HSS T3 1.8 µm, 2.1 x 50 mm column, and Waters XeVo-TQS in ESI+ mode over a 5-minute gradient. Mobile phases were ultra pure water and acetonitrile, both with 0.1% formic acid. Two ion transitions were monitored for Δ9-THC: 315.06 to 193.10 and 315.06 to 135.10. The determined Δ9-THC concentration was compared to the expected concentration and the percent differences calculated.

Results:

Separation between the two eluting peaks was dependent on the ratio of their concentrations. Ratios closer to 1:1 demonstrated better separation between the THC compounds, whereas ratios of 1:5 were more susceptible to complete co-elution. Where they were distinguished, the apex of the Δ9-THC and Δ8-THC peaks eluted 0.05 seconds apart. The range of calculated percent difference of Δ9-THC was ±6.2-45.5% of the expected concentration; all but one Δ9-THC concentration (+26.5%) was lower than the expected concentration. Reviewed casework exhibited the same elution pattern as the research samples of 0.05 seconds between compounds. Similar chromatography was observed. In all but two samples, Δ8-THC IR was less than Δ9-THC IR. For the two samples with Δ9/Δ8 concentration ratios less than 0.2, IR of Δ8-THC was greater than Δ9-THC IR. All Δ8-THC IR would pass for Δ9-THC based on analysis criteria.

Conclusion/Discussion:

With the increase in Δ8-THC appearing in samples and the difficulties caused in confirming and quantifying Δ9-THC, it is necessary to develop a method to separate and distinguish between the two THC compounds. As seen in this data, the co-elution of these compounds resulted in Δ9-THC concentration as much as 45% below the expected concentration. To further complicate Δ9-THC analysis, the ELISA method used for cannabinoid screening has greater affinity for Δ8-THC than for Δ9-THC. In the absence of Δ9-THC, Δ8-THC would be indistinguishable in confirmation. Therefore, until proper separation and identification of Δ8-THC and Δ9-THC can be achieved, quantitation of Δ9-THC should be avoided, and at best a qualitative analysis performed if a standard is used to confirm co-elution of Δ8-THC.

Prevalence of Cannabidiol and Tetrahydrocannabinol and Metabolites in Non-Regulated Workplace Drug Testing Urine Specimens

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Background/Introduction:

The non-psychoactive cannabinoid, cannabidiol (CBD), is marketed as a remedy for a wide array of medical conditions. Consequently, there is growing use of CBD containing products by the general public that may have unintended consequences to the consumer. Of concern is that CBD supplements are not subject to active regulatory oversight so their composition may deviate widely from the products' labelling and may contain tetrahydrocannabinol (THC), a psychoactive component of the cannabis plant. This puts the consumer in a buyer beware situation and presents a dilemma to the laboratory or medical review officer if the donor asserts legal CBD use as the reason for a positive drug test. To better understand the prevalence of THC, CBD and their metabolites in non-regulated workplace specimens, a pulse testing study of 4,000 urine specimens was undertaken.
Objectives:
The objective of the study was to determine the positivity rate of 11 cannabinoids and cannabinoid metabolites in non-regulated workplace drug testing specimens by screening and confirmation methods. Study analytes included CBD, Δ8- and Δ9-THC, tetrahydrocannabivarin (THCV). cannabinol (CBN), hydroxylated metabolites of THC and CBD (11-OHTHC, 7-OHCBD) and carboxylic acid metabolites of THC, CBD and THCV (Δ8- and Δ9-THCCOOH, CBDCOOH, THCVCOOH).

Methods:

In the normal course of workplace drug testing, specimens are aliquoted into test tubes and loaded onto automated instruments for screening by immunoassay (IA); after review, these aliquots are discarded. This study utilized 4,000 deidentified urine aliquots scheduled for discard to screen for 7-OHCBD and Δ 9-THCCOOH using a fast LC/MSMS technique with a 2 ng/mL cutoff and 150 second run time. Further, specimens positive using the fast chromatographic screen were reanalyzed by IA for THCCOOH at 20, 50 and 100 ng/mL cutoffs. Positive specimens were confirmed for THC, CBD and 9 other cannabinoids and/or metabolites by LC/MSMS following sequential enzymatic and basic hydrolysis.

Results:

Of the 4000 urine specimens initially screened by fast LC/MSMS (LOD=2 ng/mL) for 7-OHCBD and Δ9-THCCOOH, 434 (10.85%) specimens were identified and further analyzed by IA and LC/MSMS. The percent samples (calculated inclusively on n=4000) that screened positive by IA were as follows: 20 ng/mL cutoff, 3.40%; 50 ng/mL cutoff, 2.75%; and 100 ng/mL cutoff, 1.88%. The percent prevalence of the eleven analytes (LOD=1ng/mL) in order of highest abundance was as follows: Δ9-THCCOOH, 8.28%; 11-OHTHC, 5.28%; 7-OHCBD, 3.85%; THCVCOOH, 2.90%; CBD, 2.23%; CBDCOOH, 2.15%; Δ9-THC, 1.73%; Δ8-THCCOOH, 1.70%; CBN, 1.10%; THCV, 0.55%; and Δ8-THC, 0.05%. Four specimens screened positive by IA (20 ng/mL cutoff), but tested negative (<1 ng/mL) for Δ9-THCCOOH. Of these, one specimen contained 41.9 ng/mL of Δ8-THCCOOH (no CBD analytes), two contained low concentrations of 7-OHCBD and one contained high concentrations of CBD analytes (e.g., 1562 ng/mL 7-OHCBD). Three specimens contained concentrations of Δ8-THCCOOH in excess of Δ9-THCCOOH and were negative for CBD analytes. The percent combinations of CBD analytes in the 434 specimens in order of abundance were as follows: all negative, 62.7%; positive CBDCOOH/7-OHCBD/CBD, 13.8%; positive 7-OHCBD, 11.8%; positive 7-OHCBD/CBD, 5.3%; positive CBDCOOH/7-OHCBD, 4.6%; positive CBDCOOH/CBD, 0.7%; positive CBDCOOH, 0.7%; positive CBD, 0.5%.

Conclusion/Discussion:

Applying the method LOD of 1 ng/mL as the cutoff for all analytes, a total of 336 (8.4%) specimens had Δ9-THCCOOH concentrations and 162 (4.05%) specimens contained CBD and/or CBD metabolites. It is unsurprising that THC analyte

positive specimens were also positive for CBD analytes as both are present to varying degrees in cannabis variants. Positivity rates by IA were similar to previously published results. It is concluded that there is widespread use of cannabis and CBD products in this population.

The Power of Stakeholder Engagement in the Fight to End the Opioid Epidemic: An Overview of the APHL Opioids Biosurveillance Task Force (OBTF) Model Biosurveillance Strategy

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Background/Introduction:

Public health agencies require high quality surveillance data upon which to determine the spatial and temporal extent of non-fatal opioid overdoses (NFOO) in their jurisdictions. Clinical hospital laboratories provide information for clinical case management but not the detailed, specialized testing required to characterize drug trends and inform interventions. Specific data are needed to identify the breadth of opioids implicated in NFOOs, including a rapidly changing suite of novel fentanyl analogues. Public Health Laboratories (PHLs), working in partnership with state and local epidemiologists and forensic toxicology laboratories, are uniquely poised to provide this critical missing information. The Association of Public Health Laboratories (APHL) Opioids Biosurveillance Task Force (OBTF) Model Biosurveillance Strategy provides guidance to public health agencies interested in developing and implementing an impactful opioids biosurveillance program. The document outlines the roles of key stakeholders, fundamentals of surveillance program design, legal and policy considerations, and information on testing methods. Forensic toxicology laboratories have the unique opportunity to partner with PHLs to collaborate in an opioids biosurveillance program to better identify and understand the drug trends within each state.

Objectives:

The prevalence of opioids use and misuse, and consequently overdose incidence, can differ dramatically from state to state and within smaller geographic units. The Model Biosurveillance Strategy identifies data sources and reveals obstacles to comprehensive data collection for non-fatal drug overdoses. Current data sources do not typically provide definitive or confirmatory laboratory identification of all the substances implicated. With their expanded testing capabilities and integration within the public health system, PHLs provide unique opportunities to gather critical information to inform the development of policy, intervention and practice. This flexible Model Biosurveillance Strategy is designed to provide state, local, tribal, and territorial health departments a framework upon which they can develop a jurisdiction-specific plan.

Methods:

Each PHL will determine the appropriate testing algorithm for their respective programs in collaboration with public health partners and forensic toxicology laboratories, based on specific program objectives, available analytical instrumentation and analyst skill level. Opioids biosurveillance must provide specific drug detection information, as this aids in the early identification of emerging threats and novel substances and informs the development of appropriate public health interventions.

Results:

Health departments, in collaboration with other stakeholders, should consider short, medium, and long-term evaluation metrics as core components for assessing success of their program. These metrics should be tracked as testing capability and capacity is built. Evaluation metrics and implementation time frames are highly dependent upon the needs and

capacity of individual jurisdictions, and should be discussed early in the program development process, including input from all key that stakeholders.

Conclusion/Discussion:

Effective opioids biosurveillance programs are partnerships among experts in epidemiology, analytical chemistry, toxicology, informatics and clinical medicine. All should have input into the program design and protocol development. Biosurveillance programs can build upon and leverage existing relationships and practices developed through the Laboratory Response Network for Chemical threats and the National Biomonitoring Network. State PHLs and their public health partners should maximize community input into the program design seeking counsel from substance abuse prevention programs, medical societies, medical examiners/coroners, and epidemiologists, forensic toxicologists, state and local elected officials, and regional poison control centers. These trusted partners may facilitate relationships with emergency department personnel important to the success of a biosurveillance program. It is critical to involve epidemiologists, opioid overdose prevention specialists, and communication experts early in program design to clarify expectations and promote a common platform for internal information exchange and public messaging.

SOFTEMBER PLATFORM ABSTRACTS SESSION 8

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A Comparison of Paraphernalia Found at Death Investigation Scenes and Cause of Death in Orange County, CA from 2016-2019

Authors and Affiliations: Cody P. Woltz Orange County Crime Lab , Santa Ana, CA

Background/Introduction:

As of September of 2015, the Orange County Crime Lab's Controlled Substance (CS) section has been responsible for the analysis of paraphernalia submitted with death investigation cases. The most common drugs analyzed are methamphetamine, heroin, cocaine and fentanyl. Submissions have also started to include counterfeit tablets, most commonly Xanax™ or Oxycotin™ which contain scheduled and unscheduled drugs. Designer drugs have also been detected including common designer benzodiazepines, such as flualprazolam and etizolam. The results found by CS assist Toxicology and the pathologists by providing qualitative results within 2 to 4 weeks of receipt of evidence. These results can guide the toxicology analysis which can take significantly longer and is required before cause of death (COD) is determined.

Objectives:

To evaluate submissions of drug paraphernalia in death investigation cases from 2016 through 2019 and compare these results to the corresponding toxicology results and the COD as determined by the pathologist.

Methods:

Due to the prevalence of fake counterfeit tablets, all tablets suspected to contain controlled substances received a visual identification and instrumental analysis as of September 2015. These methods included gas chromatograph-mass spectrometer (GC-MS), liquid chromatograph-MS iontrap, and GC-infrared detector to confirm results. In addition to these tests, color and crystal tests were used to confirm substances in drug paraphernalia. The Toxicology section screened by immunoassay for four drug classes and by liquid chromatograph-quadropole time-of-flight for over 300 drugs, including some designer drugs, in central blood. Quantitative confirmations were performed by liquid chromatography with dual mass spectrometry and GC-MS for approximately 100 of the most common drugs and can be done in blood, urine or tissues. Qualitative confirmation was performed by GC-MS, GC-flame ionization detector or GC-nitrogen-phosphate detector.

Results:

The CS Section had 614 cases submitted to the lab from 2016 to 2019 that involved a death investigation. Cases ranged from 1 - 8 pieces of paraphernalia submitted for analysis. For the classical drugs, during this time, 199 (2.4%) cases had methamphetamine, 264 (42.9%) cases had heroin or 6-monoacetylmorphine, 126 (20.5%) cases had fentanyl, and 56 (9.1%) cases had cocaine. For the less common drugs, 95 (15.4%) contained other scheduled drugs 40 (6.5%) had non-scheduled drugs, and 21 (3.4%) cases had designer drugs. Of those cases, 204 (33.2%) had a combination of more than one drug. In over 75% of the cases, toxicology detected the drug found in the CS case in the postmortem samples. In over 65% of the cases, the drugs found in CS were related to the cause of death when drugs were detected.

Conclusion/Discussion:

For most postmortem cases submitted, the results between CS and Toxicology were highly consistent when the drugs were contributing to the COD. For other CODs, such as hangings or gunshot wounds, the drugs detected by CS were not as often found in toxicology. The information provided by CS assists the Toxicology section in their analysis, especially where designer drugs and uncommon drugs are concerned. If a laboratory does not have exact mass broad drug screening for toxicology, the CS results from drug paraphernalia can greatly assist in directing toxicology for the correct COD.

Concentrations of para-Fluorofuranylfentanyl in Central and Peripheral Blood from Postmortem Death Investigations

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Background/Introduction:

Over the course of the last decade, a large number of novel psychoactive substances (NPS) have appeared on illicit markets. Synthetic opioids, and particularly fentanyl analogs, attracted a lot of attention due to their involvement in adverse events and fatalities. Beginning in 2016, fentanyl analogs identifications increased in the United States and remained prevalent as several cycles of new analogs appeared on the market following scheduling actions. Fluorofuranylfentanyl was first identified in December 2018 during the decline in positivity of fluoroisobutyrylfentanyl and carfentanil. Fluorofuranylfentanyl is structurally related to furanylfentanyl by the addition of a fluorine atom to the aniline ring.

Objectives:

The objective of this study was to develop an approach for the qualitative and quantitative determination of fluorofuranylfentanyl and fluoro-4-Anilino-N-phenethylpiperidine(F-4-ANPP) (metabolite) in central and peripheral blood samples from postmortem forensic casework. Analysis was performed by liquid chromatography tandem mass spectrometry (LC-MS/MS). Following validation, quantitation was achieved by a primary LC-MS/MS method; subsequently, a secondary qualitative LC-MS/MS method was used for differentiation of isomers.

Methods:

Blood (500µL) was fortified with internal standard, buffered with 2 mL of phosphate buffer (0.1M, pH 6), and extracted by solid phase extraction using 130 mg Clean Screen® cartridges. Analysis was conducted using an Agilent 1290 Ultra High-Performance Liquid Chromatograph coupled to an Agilent 6495 Triple Quadrupole Mass Spectrometer. Quantitation was achieved using an Agilent InfinityLab Poroshell 120 EC-18®(3.0x100mm, 2.7µm) analytical column with mobile phase A 0.1% formic acid in water and B 0.1% formic acid in acetonitrile. This method allowed for chromatographic resolution of para-fluorofuranylfentanyl, ortho-fluorofuranylfentanyl, para-fluorofuranylfentanyl-3-furancarboxamide and F-4 ANPP. The method was validated in accordance with the AAFS Standards Board (ASB) standard for Method Validation in Forensic Toxicology. Qualitative determination to distinguish the para-fluoro and meta-fluoro isomer pairs was achieved by reinjecting the extracts and using an Agilent InfinityLab Poroshell 120 Chiral-V®(2.1x100mm, 2.7µm) analytical column with mobile phase A 20mM ammonium formate(pH 4) and B 0.1% formic acid in acetonitrile. Central and/or peripheral blood samples (n=29) from medicolegal death investigation cases were submitted by the Pinellas County Forensic Lab (Largo, FL). Based on case history, autopsy findings, and/or seized drug testing, a positional isomer of fluorofuranylfentanyl was suspected of being present.

Results:

All required validation parameters were assessed and determined to be acceptable, including accuracy, precision, linearity, limit of detection (LOD), limit of quantitation (LOQ), carryover, and interferences from matrix and commonly encountered drugs for all the isomers. The calibration range was 0.5-50ng/mL. The limit of detection was 0.1ng/mL. Extraction recoveries were greater than 70%. All 29 unique cases analyzed confirmed positive for para-fluorofuranylfentanyl and its metabolite F-4-ANPP. The concentrations ranged between 0.53 - 23 ng/mL in peripheral blood, and 0.66 - 36 ng/mL in central blood, and 0.56 - 32 ng/mL in peripheral and 0.56 - 46 ng/mL in central blood for the parent drug and the metabolite, respectively. Eleven (38%) were negative for other opioids. Of the eighteen cases positive for additional opioid(s), seven were prescription opioids, and three were mitragynine.

Conclusion/Discussion:
Fluorofuranylfentanyl was identified as an emerging synthetic drug of concern due to its appearance among drug overdose deaths throughout 2018 and 2019. A method was optimized and validated for the determination and quantitation

of fluorofuranylfentanyl isomers and its metabolite. In all analyzed cases the para isomer was the only isomer identified and, in many cases, this compound was the only opioid identified in the case. Twelve individuals were administered naloxone. Stimulants accounted for the most common additional substances detected, notably cocaine (34%). In one case, a 52-year-old male with history of heroin abuse was found deceased in a workplace bathroom. A syringe was found on the floor with an upside-down soda can with residue. Para-fluorofuranylfentanyl was confirmed in peripheral blood at 2.2ng/mL. Other toxicological findings included naloxone, lidocaine, trazadone, and hydroxyzine. It is important for laboratories to be aware of new emerging opioids, including these drugs in scopes of testing and developing quantitative methods for confirmation, as the synthetic opioid landscape continues to evolve rapidly.

Methemoglobin Saturation in Suicides Involving Sodium Nitrite or Sodium Nitrate

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Background/Introduction:

Sodium nitrite and sodium nitrate are inorganic salts commonly used as food preservatives and in producing dyes and fertilizers. In small doses from food, both are relatively harmless or inactivated through the heating/cooking process. However, at high doses, nitrite oxidizes the iron in hemoglobin converting it to inactive methemoglobin (MetHb) (1). Nitrate is less toxic than nitrite; however, in large enough doses, the nitrate can be reduced to nitrite by bacteria in the body, creating the same effect with MetHb (1). In general, MetHb levels <5% saturation are normal. Cyanosis, headaches, vomiting, loss of consciousness, and other toxic effects usually manifest around >20% saturation, and >70% saturation generally is considered lethal (2). Sodium nitrite or nitrate poisonings are typically accidental and rarely fatal. However, there have been three previous reports of suicidal ingestion of nitrite (3-5). As most cases are non-fatal, the MetHb concentrations provided in literature are antemortem and the utility of MetHb testing in postmortem toxicology has been debated due to stability and postmortem changes (6-7). This makes it difficult to assess postmortem MetHb saturation levels. Presented here are the MetHb saturation levels in four suicide cases involving sodium nitrite and/or sodium nitrate ingestion received by the Harris County Institute of Forensic Science between October-December 2019. An estimated postmortem interval and time between sample collection and analysis are included. Additionally, other in-house cases tested for MetHb where sodium nitrite was not suspected and published fatal cases with postmortem MetHb saturation levels are discussed.

Objectives:

We present postmortem MetHb levels for four suicides involving ingestion of sodium nitrite or sodium nitrate, one suicide involving hydrogen sulfide exposure, two cases of carbon monoxide toxicity, and one case of natural causes. Also, we compare these MetHb levels to those in published cases reports involving sodium nitrite poisoning. Being able to use MetHb as a tool to aid in determining cause of death is important in the absence of other toxicologically significant results.

Methods:

Postmortem blood specimens tested for MetHb were collected in lavender (7 cases) or gray top tubes (1 case) during autopsy and then refrigerated until sent out to NMS Labs. Blood sources were either heart (3 cases), iliac (1 case), or femoral (4 cases). When possible, the whole, unopened tube was sent to reduce exposure to air (5 cases); otherwise, an aliquot was taken from the original preserved blood tube and added to either a red top tube (1 case), or a lavender top tube (2 cases). In-house testing varied for each case based on the case circumstances and pathologist requests, but all cases did include alcohol testing for ethanol, methanol, isopropanol, and acetone and a 10-panel ELISA screen.

Results:

The four sodium nitrate/nitrite suicide cases included three males and one female, aged 17-29 years old. MetHb ranged from 31-47% saturation. No other significant toxicological findings were found, with the exception of one case where etizolam was identified. The estimated postmortem intervals were between 24-48 hours and the time from sample collection at autopsy to release of the MetHb report ranged from 6 to 23 days. For comparison, the acute hydrogen sulfide toxicity case that was also decomposed had a MetHb saturation level of 44% and an estimated postmortem interval of 80 hours. The three remaining cases, with no cause of death related to methemoglobinemia or nitrite/nitrate, had MetHb saturation levels of <1% to 20%. All three of these cases had longer estimated postmortem intervals (64-80 hours) and similar delay from collection to NMS report (7-31 days).

Conclusion/Discussion:

While MetHb has been shown to increase and decrease in samples based on storage conditions, storing EDTA preserved

blood at refrigeration temperatures appears to be the best choice for short term storage (7). The four in-house suicide cases had blood collected and stored in these ideal conditions to allow for the best interpretation opportunity. Considering few reports of postmortem MetHb levels in cases have been published, sharing these levels will aide in future methemoglobinemia cases. The small cluster of suicide cases involving nitrite/nitrate brings attention to this often overlooked, but easily accessible toxin. References 1.Baselt, R. C. (2017) Nitrite. In Disposition of Toxic Drugs and Chemicals in Man, 11th edition. Biomedical Publications, Seal Beach, CA, pp. 1539-1541. 2.Hall, A.H., Kulig, K.W., and Rumack, B.H. (1986) Drug- and Chemical-Induced Methaemoglobinaemia Clinical Features and Management. Medical Toxicology, 1, 253-260. 3.Standefer, J.C., Jones, A.M., Street, E., Inserra, R. (1979) Death associated with nitrite ingestion: report of a case. Journal of Forensic Sciences, 24, 768-771. 4.Harvey, M., Cave, G., and Chanwai, G. (2010) Fatal methaemoglobinaemia induced by self-poisoning with sodium nitrite. 5.Nishiguchi, M., Nushida, H., Okudaira, N., Nishio, H. (2015) An Autopsy Case of Fatal Methemoglobinemia due to Ingestion of Sodium Nitrite. 6.Reay, D.T., Insalaco, S.J., and Eisele, J.W. (1984) Postmortem Methemoglobin Concentrations and Their Significance. Journal of Forensic Sciences, 29, 1160-1163. 7.Varlet, V., Ryser, E., Augsburger, M., and Palmiere, C. (2018) Stability of postmortem methemoglobin: Artifactual changes caused by storage conditions. Forensic Science International, 283, 21-28.

The Rise of Counterfeit Oxycodone Tablets in Death Investigation Cases

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Background/Introduction:

Drug trends are continuing to evolve each year, with a current shift and rise in both counterfeit tablets and fentanyl use. One of the most notable observations made over the past few years is the increase in counterfeit oxycodone tablets which predominately contain fentanyl or a related compound. Of particular concern is the ability to detect these drugs in toxicology samples for death investigation cases. If a Toxicology section does not have the capabilities to screen for fentanyl, the aid of the Controlled Substances (CS) section could be instrumental in determining the correct cause of death. In 2016, the CS section of the Orange County Crime Lab began to analyze substances and paraphernalia found on or near a decedent at the time of death to assist in the investigation.

Objectives:

To evaluate the rise in counterfeit oxycodone tablets and determine the correlation between those drugs detected in these tablets and the associated toxicology death investigation case between 2016 and 2019.

Methods:

CS analysts can determine that a tablet may be counterfeit based on its visual appearance. Tablets that vary in color, thickness, and location of imprint are all good indicators that a tablet may not contain what it's commercially labeled to contain. Due to the recent prevalence of fake tablets, most tablets received by the CS section undergo a complete chemical analysis, including preliminary and confirmatory testing by GC-MS, GC-IR, and/or LC-MS/MS. The Toxicology section quantitates fentanyl in postmortem blood and tissues using DPX WAX-S extraction and LC-MS/MS with a calibration range of 0.5-32 ng/mL and a quadratic curve with a weight of 1/x 2.

Results:

During this study, a total of twenty-five cases were found to contain substances initially suspected of being oxycodone tablets. However, after analysis it was determined that 22 cases (88%) contained counterfeit oxycodone tablets instead. Of those fake tablets analyzed, 96% were found to contain fentanyl, whether on its own or combined with another substance. The most commonly received oxycodone tablet in this study had the imprint "M/30". These tablets were received in death investigation cases approximately 4x more than any other oxycodone tablet (real or counterfeit). Of those "M/30" oxycodone tablets analyzed, 100% were counterfeit and 95% contained fentanyl. Overall, there continues to be an upward trend in the submission of counterfeit oxycodone tablets in death investigation cases. In 2019 alone, there were 14 cases of "suspected" oxycodone tablets, none of which were found to contain oxycodone. This makes up more than 50% of the total cases reviewed in this study. Furthermore, 22 of the above 25 cases (88%) had toxicological findings of fentanyl in either the central blood or tissue samples. To further evaluate the correlation between counterfeit oxycodone tablets and death investigation cases, the CS and toxicology results were compared for five of the 25 cases and will be discussed in the presentation. Toxicology results showed that fentanyl was detected in the PM blood for all five of these cases with concentrations at 12.1±1.3 ng/mL, 16.9±1.8 ng/mL, 29.0±3.1 ng/mL, 29.9±3.2 ng/mL and 160±17 ng/mL.

Conclusion/Discussion:

From 2016 to 2019, oxycodone tablets submitted in death investigation cases were predominately counterfeit. Most of these fake tablets contained fentanyl, which was most frequently combined with acetaminophen. The trend of counterfeit tablets continues to be on the rise with the most cases being received in 2019. The comparison of the controlled substances results to the toxicology results showed that a correlation may exist between the items found at a scene and the substances taken by an individual prior to their death. Thus, controlled substance analyses do appear to aid in the case management of toxicology examinations.

Use of Qualitative In-House Drug and Toxicology Testing by the King County Medical Examiner's Office in Seattle, WA

Authors and Affiliations: Nicole Yarid MD* (King County Medical Examiner's Office, Seattle, WA), Leif Layman (University of Washington School of Public Health and King County Medical Examiner's Office, Seattle, WA), Katie Heidere (King County Medical Examiner's Office, Seattle, WA), Celia Simpson (King County Medical Examiner's Office, Seattle, WA), and Richard Harruff MD PhD (King County Medical Examiner's Office, Seattle, WA)

Background/Introduction:

As the opioid / drug overdose epidemic has challenged the timeliness of toxicology reporting, which in turn burdens death investigation systems and delays death certification, qualitative in-house testing has potential merit for medical examiners. As a response to this problem, the King County Medical Examiner's Office (KCMEO) in Seattle, WA has developed several in-house methodologies for toxicology and drug evidence testing. These include blood screening with an automated immunoanalyzer, urine screening with multi-drug immunoassay cups, and drug evidence testing with Raman spectrometers and a field mass spectrometer.

Objectives:

The objective of this presentation is to evaluate KCMEO's in-house screening methodologies. After this presentation attendees will be able to describe three benefits of rapid in-house testing, explain its limitations, and list four testing methodologies useful for medical examiners.

Methods:

An algorithm for probable overdose death was used to triage cases for in-house testing, contingent upon the availability of blood, urine, and drug evidence. Drug evidence collected from the scene was tested at KCMEO by ThermoFisher TruNarcTM and Rigaku ResQTM Raman spectrometers and MX908TM high-pressure mass spectrometer. Blood samples obtained at autopsy were tested by Randox Evidence MultiSTATTM automated immunoanalyzer; urine was tested using One Step Detect Multi-Panel Forensic Test urine cups. All cases had confirmatory blood testing at the Washington State Patrol (WSP) Toxicology Laboratory. Drug evidence had confirmatory testing at the WSP Crime Laboratory Chemical Analysis Unit. Blood, urine, and drug evidence results from in-house testing were compared to the results from the WSP labs. Time interval from autopsy to death certification was compared before and after in-house testing.

Results:

From 1 January 2019 to 28 April 2020, there were 604 confirmed fatal drug overdose deaths. All cases were tested at KCMEO with in-house blood and/or urine tests. Randox testing was performed on 667 cases; urine screening in 522 cases. In-house drug evidence testing was performed on 445 cases; of these, 193 (43%) were sent to WSP. Performance relative to WSP results were evaluated for four drugs: fentanyl, opiate, cocaine, and methamphetamine. Randox testing had sensitivity ranging from 85 to 100% and specificity ranging from 86 to 98%. Drug evidence testing was evaluated on the basis of MX908, Rigaku, and TruNarc results taken in aggregate and compared to final WSP crime lab test results. In this way, sensitivities were 61% for fentanyl, 88% for opiates, 90% for cocaine, and 84% for methamphetamine. Scene investigation, postmortem examination, drug evidence testing, and urine/blood testing were taken together to certify overdose deaths. In 309 deaths that otherwise would have been delayed until WSP toxicology results, death certificates were certified based on Randox results. Of these, only 8 required removal of a drug listed on the original death certificate, and no certificates were changed from overdose to a different cause of death. Over the study period, the average time from postmortem examination to certification of death due to overdose dropped from 75 days to 31 days, and the average time for completion of all autopsy reports fell from 77 days to 47 days.

Conclusion/Discussion:

In-house testing of biologic and drug evidence in suspected overdose deaths provides timely information for families, law enforcement, and public health. For medical examiners, a well-established algorithm and a minimum of two independent positive tests per drug result in expedited death certification. Furthermore, during the COVID-19 pandemic, in-house testing reduces the number of autopsies required, helping to limit the biohazard risk and conserving valuable resources.

SOFTEMBER PLATFORM ABSTRACTS SESSION 9

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A Strategy to Prioritize Emerging Novel Psychoactive Substances Using Intracranial Self-Stimulation (ICSS) in Rats

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Background/Introduction:

Novel Psychoactive Substances (NPS) are a unique threat to public health and safety, and their detection often challenges the limited resources of forensic laboratories. One way to mitigate these challenges is to efficiently allocate the available resources by predicting which threats will be the most severe. We use a technique known as intracranial self-stimulation (ICSS) in rats to rapidly evaluate the abuse liability of drugs, with the assumption that abuse potential is a major factor in determining which drugs will proliferate throughout communities. We present a strategy to classify drugs into one of four priority categories based upon their ability to facilitate (increase), depress, or be ineffectual at altering ICSS responding. ICSS is a well-established procedure that is sensitive to compounds from a variety of drug classes. This technique is demonstrated here using α-pyrrolidinohexanophenone (α-PHP), a recently-scheduled synthetic cathinone.

Objectives:

The objective of this experiment was to demonstrate the utility of abuse liability testing using ICSS in rats to classify emerging drugs into priority categories using α-PHP.

Methods:

Male Sprague-Dawley rats (n=6) were implanted with electrodes targeting the medial forebrain bundle and trained to respond by lever-press for electrical brain stimulation. Each rat received intraperitoneal administration of α-PHP (0.32, 1.0, 3.2 mg/kg), cocaine (positive control,10 mg/kg), and saline (negative control) and were allowed to respond on a lever to receive electrical brain stimulation over a range of ten frequencies. Pairs of test components were collected at 10, 30, 100, and 300 min post administration for all drug doses, and an additional time point of 1440 min was collected for 3.2 mg/kg α-PHP to evaluate the time course of effects.

Results:

Neither saline nor 0.32 mg/kg α-PHP significantly altered rates of ICSS responding. Cocaine and α-PHP (1.0 and 3.2 mg/kg) significantly facilitated ICSS responding compared to vehicle at the 10-min timepoint, indicating a rapid onset of effects. Elevated rates of responding were maintained by 3.2 mg/kg α-PHP to at least 300 min post-administration, demonstrating a long duration of action. By comparison, the effect of cocaine was only significant to the 100-min time point, even though it has a similar peak magnitude of facilitation, and returns to baseline rates of responding by 300 min.

Conclusion/Discussion:

ICSS can be used as an effective tool to evaluate the potency, time course, mechanism of action, and abuse potential of emerging NPS. The ICSS profile demonstrated by α-PHP is characteristic of a drug with high abuse potential and is similar to cocaine and other inhibitors of the dopamine transporter. This is consistent with the Drug Enforcement Administration's decision to temporarily add α-PHP to Schedule I. By our proposed classification strategy, α-PHP would fall into the Priority 1 category as a robust facilitator of ICSS responding. Although α-PHP first appeared as an NPS as early as 2014, it was not scheduled in the United States until July 18, 2019. The rapid ability of this ICSS procedure and classification system could provide one valuable source of behavioral data to inform emergency scheduling decisions earlier and provide justification for laboratories to allocate the resources to pre-emptively develop detection methods.

Pharmacodynamics and pharmacokinetics of U-47700 and its metabolites in rats

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Background/Introduction:

Novel synthetic opioids are appearing in recreational drug markets as adulterants in heroin or ingredients in counterfeit pain pills. U-47700 is an example of a non-fentanyl synthetic opioid linked to overdose deaths. Previous metabolism studies have determined the major metabolites of U-47700 as: N-desmethyl-U-47700 and N,N-didesmethyl-U-47700. No studies have been performed to determine the pharmacokinetics or biological activity of U-47700 metabolites.

Objectives:

The objective of this experiment was to evaluate pharmacokinetics and pharmacodynamics of U-47700 through an animal model. Additionally, opioid receptor experiments were performed to evaluate metabolite affinity to the μ-opioid receptor.

Methods:

Male Sprague-Dawley rats were fitted with intravenous (i.v.) catheters and subcutaneous (s.c.) temperature transponders. One week later, rats (n=6 per group) received s.c. injections of U-47700 HCl (0.3, 1.0 or 3.0 mg/kg) or saline. Blood samples (0.3 mL) were withdrawn via i.v. catheters at 15, 30, 60, 120, 240, and 480 min post injection and plasma-separated via centrifugation. Pharmacodynamic effects (hot plate latency, catalepsy, and core temperature) were assessed at each timepoint. Plasma samples (0.1 mL) were prepared via solid phase extraction and analyzed by liquid chromatography-tandem mass spectrometry using a previously validated method [Smith, J Chrom B, 2019]. The LOD were 0.05 ng/mL for U-47700 and N-desmethyl-U-47700 and 0.1 ng/mL for N,N-didesmethyl-U-47700. While the LOQ were 0.1 ng/mL for U-47700 and N-desmethyl-U-47700 and 0.5 ng/mL for N,N-didesmethyl-U-47700. Opioid receptor binding assays were carried out as described previously [Truong, Bioorg Med Chem, 2017]. Plasma pharmacokinetic data were further evaluated using APL Pharmacokinetic Modeling Program (PKMP) to determine non-compartmental parameters such as half-life (t1/2), area-under-the-curve (AUC), and maximal concentration (Cmax).

Results:

U-47700 induced dose-related increases in hot plate latency (ED50=0.5 mg/kg) and catalepsy (ED50=1.7 mg/kg), while the 3.0 mg/kg dose also caused hypothermia. Plasma levels of U-47700 rose linearly as dose increased, with maximal concentration (Cmax) achieved by 15-38 min. Cmax values for N-desmethyl-U-47700 and N,N-didesmethyl-U-47700 were delayed (80-168 min and 90-384 min, respectively) but reached levels in the same range as the parent compound. Pharmacodynamic effects were correlated with plasma U-47700 and its N-desmethyl metabolite and displayed counter-clockwise hysteresis. U-47700 displayed high affinity for μ-opioid receptors (Ki=11.1 nM) whereas metabolites were more than 10-fold weaker. Morphine (Ki=2.7 nM) was used as a positive control for the assay.

Conclusion/Discussion:

Our data reveal that U-47700 induces typical μ-opioid effects which are closely related to plasma concentrations of the parent compound. The N-desmethyl metabolite concentration increased slower than the parent compound and stayed in the bloodstream with a longer half life and later Tmax. An exceptionally slow elimination of the N,N-didesmethyl metabolite was observed, which could make this metabolite potentially a marker for U-47700 exposure in forensic case work. Given its high potency, U-47700 poses substantial risk to human users who are inadvertently exposed to the drug.

The fully automated analysis of Phosphatidylethanol in dried blood spots

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Background/Introduction:

Direct alcohol markers are widely applied during abstinence monitoring, driving aptitude assessments, and workplace drug testing. The most promising direct alcohol marker was found to be phosphatidylethanol (PEth). Compared to other markers it shows a long window of detection due to accumulation in blood. To facilitate and accelerate the determination of PEth in dried blood spots (DBS), we developed a fully automated analysis approach which is suitable for high-throughput analysis.

Objectives:

Goal was to integrate and baseline separate the two most abundant PEth homologues 16:0/18:1 and PEth 16:0/18:2 and their according internal deuterated standards within an acceptable time. Artificial and real samples have to be compared and the right source of reference material has to be found, since the regio-isomeric purity of the two fatty acid chains at the glycerol backbone can vary between suppliers.

Methods:

A validated and novel online-SPE-LC-MS/MS method with automated sample preparation and extraction using a CAMAG DBS-MS 500 system reduces manual sample preparation to an absolute minimum, only requiring calibration and quality control DBS cards being prepared manually. Furthermore, the application of the internal standards (PEth 16:0/18:1-d5 and PEth 16:0/18:2-d5) by a spray module compensates for extraction bias and matrix effects. Optimal reference material was validated by analyzing the regio-isomeric pattern.

Results:

During the validation process, the method showed a high extraction efficiency (>88%), linearity (correlation coefficient >0.9953), accuracy and precision (within ±15%) for the determination of PEth 16:0/18:1 and PEth 16:0/18:2. Within a runtime of about 7 min, the two monitored analogs could be baseline separated. A method comparison in liquid whole blood of 28 authentic samples from alcohol use disorder patients showed a mean deviation of less than 2% and a correlation coefficient of >0.9759. The comparison with manual DBS extraction showed a mean deviation of less than 8% and a correlation coefficient of >0.9666 (Luginbühl et al., Fully Automated Determination of Phosphatidylethanol 16:0/18:1 and 16:0/18:2 in Dried Blood Spots, JAT, 2019). Therefore, the automated analysis of PEth in DBS can provide a fast and accurate solution for abstinence monitoring. Additionally, the use of DBS enhances the analytes stability in contrast to the use of liquid blood. Different reference material isomer distributions led to variation in fully automated quantification of PEth in 56 authentic dried blood spot samples when a single quantifier ion was used (Gaugler et al., Variation in the relative abundance of synthetic and biologically derived phosphatidylethanol and its consequences for reliable quantification , JAT, 2020 manuscript pending).

Conclusion/Discussion:

In contrast to manual DBS extraction for PEth, no laborious sample preparation is required anymore with this fully automated setup. Our results suggest caution in ensuring the regio-isomeric composition of reference materials are well-matched with the authentic blood samples and demonstrate that both fatty acyl signals should be used in quantification of PEth in LC-SRM approaches.

The Rise of Flualprazolam

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Background/Introduction:

Flualprazolam is a designer benzodiazepine, and a derivative of alprazolam, that was first identified in March of 2018. Featured in the December 2019 edition of ToxTalk, flualprazolam detection in Sacramento DUID cases has begun to outpace alprazolam. However, due to the novelty of the drug, the available literature describing its metabolites and cross-reactivity to immunoassay screening platforms is limited.

Objectives:

The objectives of this presentation are: (1) Describe the cross-reactivity of flualprazolam, in urine samples, across three different benzodiazepine immunoassay platforms. (2) Identify metabolites of flualprazolam that are detected in urine and blood samples by LC-QTOF/MS (liquid chromatography quadrupole time-of-flight mass spectrometry). (3) Describe the observed stoichiometry of glucuronidated vs non-glucuronidated forms of parent drug and metabolites in urine samples.

Methods:

Excess urine specimens were obtained from University of California San Diego Health as well as Precision Diagnostics. Blood and urine specimens from post-mortem, sexual assault, and driving cases were analyzed at the Orange County Crime Lab by LC-QTOF/MS. Designer benzodiazepines, at 5 different concentrations, were analyzed on the Roche Benzodiazepines Plus assay, the Siemens Atellica Benzodiazepines assay, and the Thermo Fisher Scientific DRI Benzodiazepine at 3 different sites. Urine specimens were hydrolyzed with Kura B-One beta glucuronidase and were analyzed, with or without hydrolysis, by both immunoassay and LC-QTOF/MS. LC-QTOF/MS analysis was performed on a Waters Acquity Ultra Performance LC system equipped with a 1.8µm HSS C18 column. Mobile phase A (0.1% formic acid and 5mM ammonium formate in dH20) and mobile phase B (100% Acetonitrile, 0.1% formic acid) were used as solvents on a 9.5 min gradient that started at 13% B and ramped up to 50% B. Mass spectrometry data was acquired on a Xevo G2 Waters QTOF mass spectrometer. Data analysis was performed in MassLynx and UNFI software suites (Waters).

Results:

The cross-reactivity of flualprazolam relative to nordiazepam was observed to be comparable across three different immunoassay platforms. Analysis of urine specimens by LC-QTOF-MS/MS identified m/z ions of 4-hydroxy-flualprazolam, alpha-hydroxy-flualprazolam, and the glucuronidated forms of the parent drug and metabolites. Fragment ions were also identified for both parent drug and metabolites. Stochiometry of parent drug to its glucuronidated form was observed to be 1:14. While the stoichiometry of the 4-hydroxy and alpha-hydroxy metabolite to its glucuronidated form was observed to be 1:53. Analysis of 152 blood samples from known flualprazolam cases at the Orange County Crime Lab revealed a low prevalence of glucuronidated forms of either parent drug or metabolites (6% and 6.6%, respectively), and a low prevalence of non-glucuronidated hydroxy-metabolites (16.4%).

Conclusion/Discussion:

Data from the Orange county crime lab indicates a sharp rise in the number of post-mortem, sexual assault, and driving cases with detectable levels of flualprazolam. Due to the increasing prevalence of flualprazolam, we set out to characterize the cross-reactivity of the parent drug across multiple immunoassay platforms, and observed significant cross-reactivity on three different benzodiazepine immunoassay screens (Roche, Thermo Fisher Scientific, and Siemens). Immunoassay cross-reactivity was generally unaffected by the addition of beta-glucuronidase, and indicates that hydrolysis is unnecessary for the detection of flualprazolam in urine by immunoassay. Moreover, we observed one case in urine

where metabolites were detected but parent drug was not, suggesting that both parent drug and metabolites should be monitored in urine. Interestingly, we observed a low prevalence of the hydroxy-metabolites in blood specimens, and do not consider them useful for the detection of flualprazolam in blood.

Vaping in Schools: Analysis of Confiscated Vaping Devices from a Public-School System in Central Virginia.

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Background/Introduction:

Electronic cigarette use in teenage and adolescent populations has been reported at alarming rates, increasing from 19% to 35% in 12th graders who vaped from 12 months between 2017 to 2019. Risks for teenage vaping nicotine range from increased rates in ADHD and impulse control to severe lung injury, as demonstrated by the outbreak of e-vaping associated lung injury (EVALI). EVALI has been attributed to modified e-cigarette devices, adulterated e-liquids, and other chemical constituents of e-liquids. In teens, e-cigarette use has been linked to a higher prevalence in the use of drugs other than nicotine (DOTN).

Objectives:

The purpose of this study was to evaluate 62 e-cigarettes and e-liquids and three devices confiscated from grades 7 to 12 in a central Virginia public-school system.

Methods:

In a single semester, September through December 2019, e-cigarettes and e-liquids were confiscated by school resource officers (SRO)s in a public-school system in Central Virginia. SROs were instructed to complete a demographic survey of each device and assessment of suspected product tampering. Information about the student to include any symptomology was also requested. All products were bagged individually with the survey and submitted to the Laboratory of Forensic Toxicology Research (LFTR) in the Department of Forensic Science at VCU.

Results:

Of the sixty-two confiscated devices and e-liquids, 44 were pod mods, 5 were vape pens, 1 box mod, and 12 were unknown to the SRO. NJOY (40%) was the most popular brand, while JUUL was second (24%). Ten submitted samples were just e-liquids or pods with no associated device. Twenty-two of the submitted devices (36%) were reported to show evidence of tampering. Symptomology that was reported included “Student threw up”, two different accounts of “Sleepy”, “Speech is slow, always seams high”, “She was under the influence of other drugs. She was very sleepy, pinpoint pupils, slow speech and movement.”, and “Student appeared under the influence. Eyes were squinted. Student was shaking.” The highest number of confiscated samples were reported from 11th grade students with nineteen (31%), followed by 8th and 9th grades with twelve (19%) and eleven (18%) samples respectively.

Conclusion/Discussion:

The most prevalent devices confiscated were pod mods, with NJOY being the most popular brand. The 11th grade demonstrated the highest number of e-cigarettes confiscated, followed by 8th and 9th graders. The large number of modified vaping devices confiscated illustrates that these devices, even though designed to be tamper-proof, may be being altered to vape alternative e-liquids and DOTNs. Adolescents inhaling known pharmacologically active ingredients such as nicotine, chemicals that can lead to lung injury, and DOTNs pose significant challenges in school systems and communities. But, most significantly, the combination can lead to untoward legal consequences.