In January 2019, the American National Standards Institute (ANSI) published a best practice recommendation for offering opinions and testimony in the field of forensic toxicology. While the document was drafted by the Toxicology Subcommittee of the Organization of Scientific Area Committees (OSAC), the AAFS Standards Board (ASB), an ANSI-accredited Standards Development Organization, took responsibility for reviewing the document, circulating it to the general public, and adjudicating the comments received on the document. Following multiple circulations of the document to solicit public comments, the final version of the document was accepted by ANSI.

The document is intended for the subdisciplines of human performance toxicology, postmortem forensic toxicology, non-regulated employment drug testing, court-ordered toxicology, and general forensic toxicology. It is divided into sections to specifically address what is generally considered to be “appropriate” opinions and testimony by the field, as opposed to opinions and testimonies that are generally “inappropriate”.

Attendees will learn the history of the development of the document and how it relates to other ANSI/ASB published and OSAC draft documents. The key recommendations of the document will be reviewed and, where appropriate, examples will be provided. The goal is to help the attendee understand the document and so that they feel comfortable using it in their daily practice.

Free copies of ANSI/ASB Best Practice Recommendation 037: Guidelines for Opinions and Testimony in Forensic Toxicology can be obtained at www.asbstandardsboard.org.
Impaired Driving Toxicology Data Collection

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Background/Introduction: Impaired driving is a major concern in the United States and around the world. There is growing attention being focused on drug-impaired driving. However, there are insufficient data to understand the true magnitude and scope of the problem. The prevalence of drugged driving is underrepresented because many laboratories do not test for drugs when the blood alcohol concentration is above a certain predetermined level. The same core set of drugs are not tested in all cases, so data generated from different laboratories cannot be combined into a meaningful data set. Limitations with data collection systems, and variability among states, further impacts the ability to obtain quality information. These issues make it difficult to develop data driven public policies to reduce the negative impacts of impaired driving. The increase in states legalizing marijuana has exacerbated the need for reliable data to assess impacts and direct policies.

Objectives: To inform the forensic toxicology community about the limitations of the current impaired driving data systems and encourage them to collaborate to improve the quality and relevance of the toxicology data available.

Methods: The National Highway Traffic Safety Administration (NHTSA) is working to address limitations with the drug-impaired driving data in its Fatality Analysis Reporting System (FARS). FARS data provide a crucial metric for traffic safety professionals and policy makers. Current limitations make it impossible to use FARS data to determine the scope and magnitude of the drugged-driving problem or make inferences about impairment, crash causation, or compare drug and alcohol related fatal crashes. Similar issues with drug-impaired driving data exist in other data systems across the country. This presentation will highlight the limitations with current drug-impaired driving data and the need for better data, possible improvements in toxicological testing, such as conducting Tier 1 testing for all impaired driving cases, and possible improvements in data systems and data dissemination.

Results and Conclusion/Discussion: Creating consistency in the scope and sensitivity of toxicology testing in impaired driving investigations and expanding the capability of data systems to include full toxicology results, will greatly enhance the amount of quality data available. Improving the integration of toxicology testing into new/existing data systems will allow all stakeholders to have comprehensive meaningful information on the scope of the impaired driving problem and allow for the development of data driven policies to combat that problem.

Background/Introduction: The prevalence of cannabidiol (CBD)-infused products on the market has invaded most aspects of everyday life, including health and beauty, food consumables, and even dog treats. While similar in structure to tetrahydrocannabinol (THC), CBD differs widely in its properties. Most notably, it does not bind to CB1 receptors and lacks the characteristic psychoactive effects ascribed to THC. Though CBD products can be sold in CBD-only forms, more often than not, CBD marketed products also contain small quantities of THC. To add further confusion, recent literature provides conflicting reports on whether CBD can be converted to THC in acidic environments, such as those used during a forensic toxicology extraction process. This leaves the toxicologist to interpret whether positive THC results could be consistent with THC exposure, CBD consumption, or present as an artifact of the analysis method used.

Objectives: In 2016, a study by Merrick et al. noted that when exposed to an acidic environment in vitro, CBD can be transformed to THC and other cannabinoid compounds. We present here an evaluation of CBD to THC transformation as it relates to a THC hair analysis method using solid phase extraction (SPE) and gas chromatography mass spectrometry (GC-MS), a technique routinely used by our laboratory.

Methods: Drug free hair specimens were fortified with CBD solution to reach final concentrations of 50, 500, 2500, and 5000 pg/mg. The specimens were digested with strong base (1N NaOH, 1 hour). After cooling, the supernatants were brought to pH 4.5 with acetic acid and 0.1M sodium acetate buffer. The SPE columns were conditioned with methanol, deionized water, and 0.1N HCl. The supernatants were added, and each column was rinsed with deionized water and a mixture of 0.1N HCl and acetonitrile. The final elution was achieved with hexane which was subsequently evaporated to dryness. The dry residues were derivatized with BSTFA and 1% TMCS and analyzed by GC-MS in an electron ionization mode (EI). The cutoff for THC was 10 pg/mg.

Results: CBD hair concentrations of 50 pg/mg and 500 pg/mg did not produce THC levels exceeding the cutoff value of 10 pg/mg. However, the results of analysis of 2500 pg/mg and 5000 pg/mg CBD hair preparations indicate that, in the presence of acidic conditions applied throughout the analytical process, CBD may convert to THC to a small extent not to exceed 2% of the relative CBD concentration.

Conclusion/Discussion: In vitro transformation of CBD to THC using common toxicological analysis techniques has been demonstrated. Each laboratory conducting hair analysis for THC should determine the potential extent of THC formation during the analytical process applied.
S05: Fatalities Involving the Synthetic Cannabinoid, 5-Fluoro-ADB: Forensic Pathology and Toxicology Implications

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Background/Introduction: 5-Fluoro-ADB has become an increasingly popular drug of abuse as evidenced by the magnitude of confiscations reported in Europe and the increase in the number of acute intoxications and fatalities reported worldwide. The psychological and behavioral effects of 5-Fluoro-ADB share similarities with cannabis, including relaxation, euphoria, lethargy, confusion, anxiety, fear, distorted perception of time, depersonalization, hallucinations, paranoia, dry mouth, bloodshot eyes, tachycardia, nausea, vomiting, and impaired motor function. Aggressive behavior, violence and psychotic episodes have also been reported, suggesting that the effects are much more severe when compared to cannabis. Although reports describing acute and fatal intoxications involving 5-Fluoro-ADB are becoming more prevalent, very limited information has been described regarding the terminal event, autopsy findings, parent and metabolite drug concentrations in multiple drug sites, and cause of death.

Objectives: The objective is to present forty-three fatalities involving the potent synthetic cannabinoid, 5-Fluoro-ADB. Correlation between terminal event, autopsy findings, cause of death, and concentration of 5-Fluoro-ADB and its ester hydrolysis metabolite, 5-Fluoro-ADB Metabolite, in multiple blood specimens will be discussed.

Methods: Bond Elute Plexa PCX solid phase extraction columns were used to isolate the target analytes from postmortem blood specimens. Extracts were submitted to an Agilent 1290 HPLC system coupled to an Agilent 6460 electrospray triple quadrupole mass spectrometry for analysis. Separation was achieved using an Agilent PFP Poroshell 120 (4uMx3x50mm) analytical column with an aqueous mobile phase of 5mM ammonium formate with 0.1% formic acid in LC-MS grade water, and an organic mobile phase of LC-MS grade acetonitrile with 0.1% formic acid. The linear range of the method is 0.010 – 10 ng/mL for 5-Fluoro-ADB and 10 – 500 ng/mL for 5-Fluoro-ADB Metabolite, with a limit of detection of 0.010 ng/mL and 0.50 ng/mL, respectively.

Results: Central blood concentrations ranged from 0.010 to 2.2 ng/mL (average: 0.34 ng/mL) for 5-Fluoro-ADB and 2.0 to 166 ng/mL (average: 41 ng/mL) for 5-Fluoro-ADB Metabolite. Peripheral blood concentrations ranged from 0.010 to 0.77 ng/mL (average: 0.15 ng/mL) and 2.0 to 110 ng/mL (average: 21 ng/mL) for 5-Fluoro-ADB and 5-Fluoro-ADB Metabolite, respectively. 5-Fluoro-ADB central to peripheral blood concentration ratios (C/P) greater than 1 was reported for 58% of the cases, whereas 71% of the cases resulted in 5-Fluoro-ADB Metabolite C/P greater than 1. Non-specific findings at autopsy included pulmonary congestion and edema and aspirated gastric contents; cardiac weights measured for the decedents were between 280 – 710 grams, with lung weights varying between 270 – 1460 grams. The volume of gastric contents recorded for 78% of the cases was greater than 100 mL.

Conclusion/Discussion: The low concentrations calculated confirm the necessity for sensitive analytical techniques to identify 5-Fluoro-ADB in postmortem blood specimens. 5-Fluoro-ADB Metabolite is present in much greater concentrations in blood than 5-Fluoro-ADB, suggesting its use as a marker for synthetic cannabinoid abuse. Furthermore, the usefulness of screening central blood is evident based on its increased concentration, possibly due to postmortem redistribution, when compared to peripheral blood concentrations. Combining the toxicological and pathological findings, it can be hypothesized that individuals with cardiomegaly may be more susceptible to the adverse effects of 5-Fluoro-ADB. In addition, the physical demand on cardiac output in the post-prandial period can precipitate a dysrhythmia and sudden death.

Keywords:
5-FLUORO-ADB, SYNTHETIC CANNABINOIDS, POSTMORTEM REDISTRIBUTION
S06: Interpretation Issues in a Death Involving Gliclazide, an Oral Hypoglycemic Drug

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\textbf{Background/Introduction:} Diabetes mellitus type 2 (non-insulin dependent diabetes) is a chronic metabolic syndrome. Major symptoms include hyperglycemia and aberrant metabolism of proteins, fats and carbohydrates. Control of blood glucose concentrations is possible by appropriate medication. Several classes of anti-diabetics are available, including sulphonylureas. Treatment with sulphonylureas is potentially life threatening by hypoglycemia. Gliclazide, a member of this class, is on the WHO list of essential medicines. Although its used by millions of patients around the world, little postmortem data is available for gliclazide.

\textbf{Objectives:} An unexpected death of a 46 year-old woman presenting a type II diabetes was observed and the Prosecutor requested an autopsy followed by toxicological investigations. Autopsy findings (labial ecchymosis, multi viscera congestion, asphyxia syndrome, moderate cerebral edema) were in accordance with a possible hypoglycemia death. Toxicological analyses, performed 6 weeks after the death (while specimens were stored at + 4°C), revealed the presence of gliclazide in femoral blood at 2.2 mg/L, which is in the range of published therapeutic concentrations. During a meeting with the pathologist, 3 possible explanations were discussed: 1. unknown cause of death (other than a toxic death), 2. death due to gliclazide which could have been degraded due to chemical instability (at the time of death the blood concentration could have been much higher), and 3. blood concentration was enough to produce fatal hypoglycemia in a non-observant patient. The objectives of this presentation are to discuss, based on literature survey and additional tests, these possible scenarios.

\textbf{Methods:} During autopsy, cardiac blood, femoral blood, vitreous humor, gastric content and hair (6 cm, dark) were collected. Gliclazide, identified during a comprehensive screening, was tested in the various specimens by LC-MS/MS after acid extraction. To document chemical stability, 20 mL of blood were spiked with gliclazide for a final concentration at 10 mg/L, then divided into 1.2 mL vials, kept at + 4 °c and – 20 °C. Specimens were tested over 3 months.

\textbf{Results:} Blood alcohol was negative. Toxicological screening detected gliclazide, which was then quantified using a MRM method. The following concentrations were measured: femoral blood (2199 ng/mL), cardiac blood (1949 ng/mL), vitreous humor (36 ng/mL), and gastric content (< 10 ng/mL). Hair tested also positive in the 3 x 2 cm segments, at 7, 8 and 3 pg/mg. No other drug, including pharmaceuticals, drugs of abuse and NPS was identified. After 3 months, a final loss of about 35 % (at – 20 °C) and 70 % (at + 4 °C) of gliclazide was observed, indicating chemical instability.

\textbf{Conclusion/Discussion:} The major issue in this case is the therapeutic concentration of gliclazide detected in the femoral blood of the victim. Although a fatal gliclazide concentration has never been described in the literature, the question was to evaluate possible death in such circumstances. Testing for gliclazide stability in whole blood over a period that matches the delay of the toxicological analyses did not demonstrate massive instability (loss of about 30 % after 6 weeks storage at + 4 °C). On the opposite, segmental hair results, with low concentrations over 6 months, were highly indicative of non-compliant use of the medicine. In subjects under gliclazide therapy, hair concentrations (n=6) were in the range 550-1200 pg/mg, much higher than what was measured in the hair of the victim. Although not studied nor reported, one can anticipate good gliclazide stability in hair, as it is the case for all other drugs when the specimen is stored dry at room temperature. It was therefore concluded by the pathologist that the cause of death of the subject was an inappropriate use of gliclazide, a drug that could be responsible of a fatal hypoglycemia, even at normal therapeutic concentration.
S07: Δ-9-Tetrahydrocannabinol Distribution in Central Blood, Peripheral Blood, and Brain Tissue

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Background/Introduction: Once ingested, Δ-9-tetrahydrocannabinol (THC) quickly moves out of the blood and into fatty tissues, including the brain. As a result, concentrations in the blood may not represent concentrations in the brain, making correlation to impairment difficult, at best. Postmortem redistribution may also complicate interpretation of the involvement of the drug in the death. Postmortem blood and tissue samples were analyzed and evaluated for individuals who died in traumatic collisions while driving a motor vehicle and compared with individuals who died of other causes.

Objectives: The objective of this research was to quantitate THC in brain tissue and compare concentrations to corresponding central blood and peripheral blood samples.

Methods: Forty-three THC positive central blood samples were selected. Twenty-one of the deceased individuals were drivers in fatal automobile collisions, while the other 22 died of other causes (natural, homicide, suicide, etc.). When available, central cardiac blood, peripheral blood, and brain tissue were analyzed.

Blood was sampled at 150 µL and homogenized brain tissue (2x) at 0.6 g. The sample was mixed with 0.1% formic acid and deuterated internal standard (THC-d3) then added to an Isolute SLE+ column. The extracts were eluted with 70:30 hexane:ethyl acetate. Samples were dried with heated air (50 - 70 °C) and reconstituted in mobile phase.

Separation occurred on a Waters Aquity UPLC with HSS T3 column using aqueous and organic phases of 100% water and acetonitrile, each with 0.1% formic acid. The LC method consisted of a 5-minute gradient. A Waters XeVo-TQS collected MRM data in ESI+ mode with two ion transitions. The quantitative range is 1-100 ng/mL for THC on a quadratic curve, with the administrative LOD set at the LOQ. This method was previously validated with SWGTOX validation standards.

Ratios for THC concentrations were calculated for all blood and brain tissue combinations.

Results: Among driver cases, eight of 21 peripheral bloods and seven of 14 brain samples tested had concentrations greater than the central blood concentrations. All brain samples tested were positive for THC. In the non-driver cases, 13 of 21 peripheral bloods and eight of 22 brain samples tested had concentrations greater than the central blood concentrations. In some cases, brain tissue did not contain quantifiable amounts of THC when it was detected in central blood. The table below summarizes the observed ratios between all sample types.

<table>
<thead>
<tr>
<th>THC Concentration Ratios</th>
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<tbody>
<tr>
<td>Central/Peripheral Blood; Central Blood/Brain Tissue; Peripheral Blood/Brain Tissue</td>
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<tr>
<td>n=</td>
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<tr>
<td>---</td>
</tr>
<tr>
<td>Drivers</td>
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<td>Non-Drivers</td>
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<td>Total</td>
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Conclusion/Discussion: Interpretation of postmortem THC concentrations is difficult due to the nature of the drug. Variations in sample collection at autopsy may also influence postmortem concentration changes. In this study, postmortem blood and tissue samples were analyzed for THC to determine if any patterns in distribution were observed when comparing concentrations. Stronger conclusions could not be drawn due to the lack of information regarding impairment and the historical cannabis consumption for the diseased. There were also limitations in the number of cases with all three sample types available for comparison. Among the driver group, all tested brain tissue was positive for THC, indicating a possibility these individuals were affected by THC at the time of the fatal collision.
S08: Distribution of Synthetic Opioids in Postmortem Blood, Vitreous Humor and Brain

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Background/Introduction: In the US, the use of synthetic opioids has become an increasing health issue with thousands of overdose deaths observed since 2013. With the high mortality rate associated with these substances, postmortem analyses and interpretation of synthetic opioids has become extremely important. However, due to the novelty of these compounds, the available data are limited and provides challenges to toxicologists.

Objectives: (1) Develop and validate analytical methods for the determination of fifteen synthetic opioids (3-methylfentanyl, 4-ANPP, 4-methoxybutyrylfentanyl, acetylfentanyl, butyrylfentanyl, carfentanil, fentanyl, furanylfentanyl, isobutyrylfentanyl, MT-45, norfentanyl, p-fluorobutyrylfentanyl, U-47700, valerylfentanyl and W-18) in vitreous humor and in brain tissue; (2) Investigate the postmortem distribution and detectability of synthetic opioids within blood, vitreous humor and brain samples.

Methods: 0.5-mL vitreous humor and 3g of brain tissue homogenized in water (diluted 1:3, w/w) were extracted by mixed-mode cation exchange-reversed phase solid phase extraction. Extracts were analyzed by LC-MSMS. The internal standards employed were fentanyl-d₅ and norfentanyl-d₅. Chromatographic separation was performed by reversed-phase (Agilent Poroshell 120 EC18, 2.1 x 100mm, 2.7µm) with 0.1% formic acid in water and in acetonitrile as mobile phases in gradient mode, with a total run time of 19min. Data were acquired with ESI+ in dynamic multiple reaction mode (dMRM), monitoring two transitions per compound. The methods were validated following SWGTOX guidelines. Fifty-eight authentic case samples from the New York City Office of the Chief Medical Examiner (NYC-OCME) were analyzed. These cases were positive for at least one of the analyzed opioids, and all three matrices were available. The blood sample sources included femoral (n=42), iliac (n=2), subclavian (n=3) and cardiac (n=11).

Results: Vitreous humor was cross-validated with a previously validated blood method. All quantitative analytes had a limit of quantification of 0.1ng/mL. Quantitative analytes had acceptable bias (-5.8 - 15.9%) and precision (CV, 0.6-12.4%) at all three levels of QCs (0.5, 8, and 80ng/mL, n=15). Significant matrix effects were exhibited for MT-45, p-fluorobutyrylfentanyl, valerylfentanyl, and W-18 (up to -60.4%, CV<20%, n=10). All analytes demonstrated extraction efficiencies 65.6-84.1%, except for norfentanyl and norfentanyl-d₅ which were 29.5-31.7%. The brain extraction method had a linear range of 0.1-100ng/g. Quantitative analytes had acceptable bias (-10.1 - 11%) and precision (0.6-14.3%) for all levels of QC (0.5, 8, and 80ng/g, n=15). The majority of analytes had matrix effects <20%. Most analytes had extraction efficiencies of 50.1-80%, except for 4-methoxybutyrylfentanyl, butyrylfentanyl, furanylfentanyl, MT-45, and norfentanyl that ranged from 21.1-43.4%. No long-term stability studies were performed.

Six of the fifteen analytes (4-ANPP, acetylfentanyl, fentanyl, furanylfentanyl, norfentanyl, U-47700) were detected in the authentic cases. Concentrations were within the 0.1 to 100ng/mL or ng/g across all three matrices, with only concentrations of acetylfentanyl and U-47700 exceeding the upper limit. The highest concentrations were observed in brain (except norfentanyl), followed by blood and vitreous humor. Most analytes were detected in all three matrices in a given case (from 96% of cases for fentanyl to 39% for ANPP). 4-ANPP had the highest percentage of cases in which it was only detected in blood (24%), as well as the highest percentage of cases in which the analyte was only detected in blood and brain (22%).

Conclusion/Discussion: We developed and validated two sensitive and specific methods for the detection of fifteen synthetic opioids in vitreous humor and brain samples. The synthetic opioids in this study displayed a higher affinity for brain tissue when compared to blood and vitreous humor as presented by the larger concentrations detected in brain, except for norfentanyl. Brain tissue and vitreous humor were demonstrated to be viable alternatives in detecting the presence of synthetic opioids in place of blood.
Introduction: Gabapentin (Neurontin) is a widely prescribed drug, approved for use as an anticonvulsant for epileptic seizures and for management of post-herpetic neuralgia, though frequently prescribed for any number of off-label purposes. While gabapentin is structurally similar to the neurotransmitter GABA, it does not bind to or activate GABA receptors, and its mechanism of action remains relatively unknown.

Despite not having typical CNS depressant effects, gabapentin is widely used and abused in conjunction with illicit opioids. Data from 2015 shows gabapentin is present in 26% of North Carolina drug overdose deaths, with 96% of those cases including an opiate or opioid. Gabapentin use continues to grow, and since 2015 North Carolina has seen an 18% increase in gabapentin-positive cases.

More cases however, lead to more potential problems. With a volume of distribution near 1, gabapentin does not exhibit classical postmortem redistribution, but is not exempt from other anomalies and preanalytical artifacts. At NC OCME the toxicology lab screens for gabapentin in a central blood specimen and confirms on a peripheral blood specimen. Most of the time the screen and confirmation results closely match, however there are times where the peripheral is much greater. Since gabapentin is eliminated intact by renal excretion, the possibility of diffusion from the bladder into the iliac vein exists and could significantly increase gabapentin concentrations after death. These discrepancies, if left unchecked, could result in erroneous means and manner of death.

Objectives: The goal of the presentation is to provide examples of disparate central-to-peripheral gabapentin concentrations and how to interpret that information in the setting of a postmortem medicolegal case.

Methods: Gabapentin is screened by a validated multi-analyte targeted assay employing a high-resolution, accurate mass Thermo Orbitrap LC-MS/MS with a cut-off for confirmation of 1.0 mg/L. Confirmation and quantitation is achieved by a validated LC-MS/MS method in blood using 0.1 mL of specimen and using gabapentin-d_{10} as an internal standard. Positive electrospray ionization on a Thermo TSQ triple quadrupole LC-MS/MS monitors two transitions for each analyte with identification criteria based upon retention times and ion ratios. A whole blood linear calibration curve of 1.0 – 50 mg/L, as well as matrix matched controls is included with each batch of specimens. Our internal database was searched for cases where the gabapentin concentration of the peripheral specimen greatly exceeds the central.

Results: A few select cases are presented below. Also, to be presented are supporting instrumental information, additional case studies, and considerations for postmortem interpretation.

Conclusion: Preanalytical artifacts arising from diffusion from the bladder to the iliac vein could result in elevated gabapentin concentrations. If left unnoticed, these elevated concentrations could lead to erroneous means and manners of death. If screening on a central specimen and quantitating on a peripheral specimen it is important to compare the two concentrations, particularly when an iliac specimen is used. Great care must also be taken when a peripheral specimen is used for both screening and confirmation, particularly if gabapentin is to be invoked in the means and manner of death.

Keywords: Gabapentin, postmortem toxicology, preanalytical artifacts
Background/Introduction: There were 70,237 drug overdose deaths in 2017, with more than two-thirds involving opioids. The current opioid overdose epidemic has been characterized by three waves: deaths involving prescription opioids (1990s), followed by increases in deaths involving heroin (starting in 2010), and most recently deaths involving fentanyl (starting in 2013). The fentanyl wave of the epidemic has predominantly involved illicitly manufactured fentanyl, but has also included deaths attributable to fentanyl analogs, in combination with fentanyl, with each other, or alone.

Objectives: To present trends in drug overdose deaths involving fentanyl and fentanyl analogs among jurisdictions funded by CDC’s Enhanced State Opioid Overdose Surveillance (ESOOS) program.

Methods: ESOOS currently funds 32 states and Washington, DC to report data on nonfatal and fatal opioid overdoses. Data on fatal opioid overdoses that were unintentional or of undetermined intent are collected within the State Unintentional Drug Overdose Reporting System (SUDORS) and reported to CDC biannually with a lag of 8–13 months after the date of death. Funded jurisdictions abstract data from death certificates and medical examiner/coroner reports, including complete postmortem toxicology test results. Data on opioid overdose deaths that occurred during July 2016–June 2018 were analyzed to determine the number of unintentional or undetermined intent overdose fatalities that involved fentanyl and fentanyl analogs.

Results: There were 40,243 opioid overdose deaths reported by 28 jurisdictions during July 2016–June 2018, with the number of reporting jurisdictions varying over time. Across all jurisdictions and periods, fentanyl was detected in 25,093 (62.4%) deaths and ≥1 fentanyl analog was detected in 8,316 (20.7%) deaths; either fentanyl, ≥1 fentanyl analog, or both, were detected in 28,027 (69.6%) deaths. All 28 states had ≥1 death each with fentanyl and any fentanyl analog detected. The most commonly detected fentanyl analogs were acetylfentanyl (n=3,089, 7.7%, 27 states); carfentanil (n=2,205, 5.5%, 19 states); cyclopropylfentanyl (1,062, 2.6%, 22 states); a combined group of “fluorofentanyls” (because of identification issues, this includes fluorobutyrylfentanyl, 4/para-fluorobutyrylfentanyl, fluoroisobutyrylfentanyl, and 4/para-fluoroisobutyrylfentanyl) (n=1,023, 2.5%, 22 states); and furanylfentanyl (n=784, 2.0%, 22 states). Fourteen additional fentanyl analogs were detected in ≥1 death. During July 2016–June 2018, there were sequential peaks in deaths with carfentanil, cyclopropylfentanyl, and furanylfentanyl detected, at 246, 140, and 113 deaths, respectively, followed by drop-offs to fewer than 50 deaths each by June 2018. There was an initial peak (113 deaths) and drop-off in detection of “fluorofentanyls;” however, there was a secondary peak with 94 deaths in May 2018. Acetylfentanyl was detected in increasingly higher numbers of deaths over time, up to a high of 322 deaths in June 2018.

Conclusion/Discussion: Fentanyl and/or a fentanyl analog were detected in over two-thirds of opioid overdose deaths reported to SUDORS during July 2016–June 2018. Most of the detected analogs followed a pattern of increasing detection over time to a peak, followed by a drop-off to a few deaths by June 2018. This decrease coincided with the temporary scheduling by DEA of all fentanyl-related substances in February 2018. Opposing this trend, the category of “fluorofentanyl” appeared to have a secondary peak, and acetylfentanyl was detected in increasing numbers of deaths over time through June 2018. This supports some existing evidence of acetylfentanyl as a potential contaminant in the production of illicitly-manufactured fentanyl, in addition to being a distinct analog. SUDORS represents a unique data source to examine complete postmortem toxicology test results of opioid overdose deaths (not limited to those substances determined to have caused the death). Continuing to monitor trends in the detection of fentanyl, fentanyl analogs, and other new psychoactive substances over time can help elucidate drug market trends and identify opportunities to prevent overdose deaths.
Background/Introduction: Methyl ether, also referred to as dimethyl ether, is a flammable compound with a boiling point of -11.2°F (-24°C). It is used as a refrigerant, an aerosol propellant, and as an alternative fuel. It is also used in the synthesis of other compounds, such as dimethyl sulfate and trimethylxonium tetrafluoroborate. The authors are aware of only one postmortem report involving inhalation of methyl ether. In that case, the substance was detected in the brain of a decomposed body. Very little is known about the concentration of methyl ether in tissues and fluids in cases in which the chemical was implicated in the death.

Objectives: The objectives of this study were to determine the concentration of methyl ether in four postmortem cases where volatile compounds were thought to play a role in the death; methyl ether concentrations were also studied in two cases of DUID where methyl ether was detected along with other drugs.

Methods: A screen for volatile substances was requested in four postmortem and two DUID cases. Blood (0.5 mL) was screened for volatile compounds by dual column head space gas chromatography and subsequently identified by gas chromatography/mass spectrometry. Methyl ether was quantified by use of a 6-point calibration curve prepared in a blood matrix. Calibrators ranged from 940 ng/mL to 47,000 ng/mL. The internal standard was 1,4-dioxane. Linear regression with a second order curve with 1/x weighting was used and typically yielded $r^2$ values greater than 0.999. A high and a low control were assayed with each batch. Specimens were analyzed with no dilution and with a 1:9 dilution. CV’s for the high and low control are <10%. Briefly, the method is as follows. Blood, 0.5 mL, and internal standard was added to a 20 mL headspace vial and immediately sealed. Calibrators and controls were spiked with appropriate amounts of dimethyl ether. The vials were incubated at 70°C for 20 minutes then sampled and injected. The split ratio was 15:1. The column was a 30 meter X 0.32 mm ID WAX column with a film thickness of 0.50 μm. The oven was immediately programmed from 40°C to 60°C at 4°C/min. A FID was used as the detector.

Results: The four postmortem cases had methyl ether blood concentrations that ranged from 200,000 ng/mL to 770,000 ng/mL (mean 488,000 ng/mL, median 490,000 ng/mL) as compared to the DUID law enforcement cases where concentrations of 3,100 and 19,000 ng/mL were detected in blood. Three of the four postmortem cases had no other significant findings while the other case had 1,1 difluoroethane (DFE) present at a concentration of 180,000 ng/mL along with 39 ng/mL of 7-aminoctazepam, 1.0 ng/mL of Delta-9-THC, and 10 ng/mL of buprenorphine. Significant findings in addition to methyl ether were present in the two DUID cases. One case had 1,700 ng/mL of DFE and 0.55 ng/mL of Delta-9-THC in addition to 3,100 ng/mL of methyl ether. The second law enforcement case had findings of 19,000 ng/mL of methyl ether, 18 ng/mL of Delta-9-THC, 9.7 ng/mL of 11-hydroxy Delta-9-THC, and 10 ng/mL of bupropion.

Conclusion/Discussion: In the cases presented in this study, the concentrations of methyl ether in postmortem specimens were significantly higher than those detected in the law enforcement cases. However, there is always a time delay in collection of specimens in DUID cases, which may account for the lower concentrations. Interpretation of the methyl ether concentration in the DUID cases is also complicated by the presence of significant findings for other impairing substances. Unfortunately, no information was submitted concerning the observed behavior of the DUID subjects. The presence of methyl ether can be detected in routine screening for volatile compounds. In addition, laboratories should be cognizant of an early eluting peak in routine screens for ethanol, as this peak was subsequently identified as methyl ether in our postmortem cases.
Background/Introduction: Isopropanol is a simple alcohol commonly used as an antiseptic and household disinfectant. Persons suffering from alcohol addiction or those with suicidal intentions may consume it as an ethanol substitute due to its ease of access. It acts as a central nervous system depressant, likely affecting the brain stem, leading to respiratory depression and circulatory collapse. Isopropanol is rapidly absorbed, widely distributed, and metabolized to acetone by alcohol dehydrogenase. It is roughly twice as toxic as ethanol though deaths are rare, possibly owing to proper emergency care. In the case presented here, a 48 year old male, who was displaying increasingly bizarre behavior in the weeks leading up to the incident, forced his 82 year old female landlord to drink rubbing alcohol. He proceeded to set her on fire because he believed she was possessed by the Devil. Her body was recovered from the bedroom in her home by arson investigators with burns covering 100% of her body. The autopsy revealed that the stomach contained 14 red-orange tablets presumptively determined to be nifedipine through drug chemistry analysis as well as foam in the respiratory tract with congested lungs and aspirated gastric contents. No evidence of strangulation or soot in the airway was present. Routine toxicology testing revealed the presence of ethanol, isopropanol and acetone and a sub-therapeutic level of nifedipine. The postmortem carboxyhemoglobin blood saturation percentage was below clinical significance.

Objective: The objective of this case study was to quantify the amount of isopropanol, ethanol and acetone in the heart blood and urine of an individual suspected to have been compelled to consume rubbing alcohol. Due to the condition of the body, femoral blood and vitreous humor was not available for testing.

Method: Samples were analyzed using dual column headspace gas chromatography with flame ionization detection (HSGC-FID). 100 µL of sample was diluted with 700 µL 0.02 % n-propanol in a 20 mL headspace vial. Vials were heated to 65°C and agitated for 2 minutes prior to injection on an Agilent 7890A GC using a gas tight syringe heated to 70°C. The columns used were DB-ALC1 and DB-ALC2 with a run time of 4.5 minutes at 40°C isothermal. Confirmations of volatile positive samples were performed on a second day.

Results: Blood and urine samples collected at autopsy were each assayed and the following volatile concentrations determined:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ethanol (g/100 mL)</th>
<th>Isopropanol (g/100 mL)</th>
<th>Acetone (g/100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Blood</td>
<td>0.046 ± 0.004</td>
<td>0.175 ± 0.010</td>
<td>&lt; 0.025</td>
</tr>
<tr>
<td>Urine</td>
<td>ND</td>
<td>&lt; 0.025</td>
<td>ND</td>
</tr>
</tbody>
</table>

The ethanol was likely a component of the rubbing alcohol, and the acetone a metabolite of isopropanol. No vitreous fluid was available to ascertain glucose levels in order to exclude diabetic ketoacidosis as a source of acetone due to thermal damage. The decedent had no medical history of diabetes mellitus, however.

Conclusion/Discussion: This is the first known report of homicide by isopropanol ingestion. In cases of suicidal/accidental isopropanol ingestion concentrations of acetone are much higher than isopropanol due to the shorter half-life of isopropanol versus acetone. Here, a low concentration of acetone was found in the blood and none in the urine. The blood level of nifedipine was below toxic level despite the presence of numerous tablets within the stomach; these findings suggest a quick demise due to isopropanol ingestion. In addition, alcohol dehydrogenase preferentially oxidizes ethanol over isopropanol; this would have extended the half-life of isopropanol, thus increasing its toxicity.
S13: Ivabradine, a Non-Fatal Cardiotoxant Overdose and Its Presence in an Unexplained Case

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Background/Introduction: Ivabradine was approved in 2015 for the treatment of inappropriate sinus tachycardia and other electrophysiological disorders. It has a unique mechanism of action on the sinoatrial (SA) node of the heart (natural pacemaker) where it antagonizes or has a negative chronotropic effect on the funny channel (If) when If is in the open state, resulting in slowing of the heart rate. Ivabradine is only selective to the SA node and does not target the atrioventricular (AV) node or neurohormonal system, as other medications do (anti-arrhythmic, beta and calcium channel blockers). In an ivabradine overdose, an individual will not go into cardiac arrest but will experience bradycardia. The slowest an individual’s heart rate can reach is ~30 – 60 bpm since only the SA node is affected. Presented is a method and determined plasma/serum concentrations of ivabradine in two cases. The first case is an intentional overdose case involving ingestion of fifty 5mg pills. On arrival, vital signs and neurologic exam were unremarkable. Within 30 min, her heart rate decreased to 31 bpm, but remained normotensive with no change in mentation. Two additional bradycardic episodes occurred, but were treated. Thirty-six hours post ingestion, her heart rate was 67 bpm. The second case involved an individual who presented with symptomatic sinus bradycardia over 1-2 weeks. She was previously prescribed ivabradine for sinus tachycardia, which she reportedly had not taken for several days. The bradycardia persisted with heart rates in the 40-bpm range, but responded to exertion. Due to a subsequent event with a syncopal episode, she was hospitalized for 3 days. Ivabradine had a reported half-life of 2 – 11 h, her clinical presentation indicated its presence, reported use did not.

Objective: To develop and validate a method for the analysis of ivabradine in overdose and unexplained cases.

Methods: Ivabradine and its isotopically labeled standard (50 ng/mL) were extracted using a 1 mL aliquot of plasma/serum and extracted with 4 mL of ethyl acetate, vortex mixed for 3 min, and centrifuged at 8000g for 10 min. The organic layer was removed and evaporated under a stream of nitrogen gas in a dry-bath at 40°C. The residue was dissolved in 200 mcL of mobile phase, vortex-mixed for 1 min, and centrifuged at 8,000g for 5 min. then transferred to the LC-MS/MS system where 5 mcL was injected for analysis. Linearity was assessed from 1 to 100 ng/mL. Validation controls were prepared at 1, 5, 30, 75, and 750 (dilution 1:9) ng/mL Storage stability was determined at 5, 30, and 75 ng/mL for 1 and 2 days, and 1 month at 5°C, benchtop for 72 hours and 3 freeze-thaw cycles.

Results: The overdose patient’s plasma/serum ivabradine concentration was determined in three different blood collection tube types (EDTA, serum separator and plasma separator) upon arrival and at 3 h post-admission. The arrival concentrations were 525, 464, and 460 ng/mL, respectively, and 3 h post-admission concentrations were 130, 120, and 110 ng/mL, respectively. The unexplained patient’s serum ivabradine concentrations at admission, 12 h and 36 h post-admission were 27, 11, and 6 ng/mL respectively. Intra and inter-run precisions were < 15%, determined bias was < 7%, and QC was stable at all testing conditions.

Conclusion/Discussion: The ivabradine concentrations determined in the overdose patient and unexplained patient were approximately 50 and 2 times the expected therapeutic concentration, respectively. The method was robust and reliable for the analysis of ivabradine in serum or plasma specimens.

This work was supported in part by the National Institutes of Health grant P30DA033934.
S14: Broad Analyte Screening Based on High Resolution Accurate Mass Technology for Postmortem Forensic Applications

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Introduction: Traditional screening methods in postmortem toxicology have relied on drug class screening utilizing immunoassay-based technology, chromatographic methods, and analyte-specific approaches using LC-MS/MS or GC-MS with library matching. Limitations of these methods include non-specific identifications, time-consuming development and validation, and limitations in analyte scope. Application of high-resolution accurate mass (HRAM) technology enables a screening method that allows broad analyte scope, fast development and validation, and robust identification of analytes. The developed method is based on a combination of targeted and unknown analyte matching, using data dependent MS² (ddMS²) collection.

Objectives: Develop an analytical method based on HRAM and library matching to enhance screening capabilities for post-mortem drug identification.

Methods: Blood and serum samples were prepared by phospholipid depletion (Phree, Phenomenex) and a dilution protocol for urine. Analysis is carried out with a Thermo Scientific Vanquish UPLC and Q Exactive Orbitrap Mass Spectrometer equipped with a biphenyl column (2.1 X 50 mm, 2.6 µm). The method was validated by analysis of >150 analytes to determine interferences, carryover, limit of identification, and identification accuracy (or measurement of error). Internal standards are used to evaluate extraction efficiency with an RSD limit of <20%. Parallel studies were conducted in whole blood, serum, and urine and the results were verified with an LC-QqQ screening and confirmation methods. The resulting data is matched to a verified library with known performance which includes matching parameters on retention time, isotope pattern matching, and exact parent and fragment masses. Matching is also conducted with an in-house modified library based on the Thermo Scientific MZ Vault spectra program.

Results: Sample preparation is carried out with the Phenomenex Phree solid phase phospholipid removal columns by mixing the matrix in a 4:1 ratio with acidified organic buffer and eluting the mixture directly from the column with positive pressure. The samples are dried and reconstituted in methanol for analysis. This system generates percent recoveries of >80% for a broad range of analytes with one sample preparation procedure; simplifying the laboratory workflow process and allowing complete processing in less than 1 hour. The verified library is analyte specific and able to detect and identify analytes with limits of identification of typically 5-10 ng/mL and identification accuracy of >93%. The subsequent data files are matched to a verified library of >150 analytes and an MZ vault library of >1500 compounds covering illicit, novel psychoactive and therapeutic drugs (Figure 1).
Conclusion/Discussion: We developed a single screening method with a simple sample preparation, a broad scope of analytes, and a fast validation process. The verified and MZ Vault libraries can be expanded in house as additional substances emerge or are added to the market. Positive identification of analytes in patient samples are generated by matching 8 criteria; retention time, peak shape, mass error, isotope pattern matching, number of fragments, library matching, library score, and signal to noise. These criteria have proven to provide high confidence in results.
S15: Identifications and Quantification of Exogenous Insulin Analogs in Postmortem Specimens

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Background/Introduction: The detection of exogenous insulin in postmortem samples is of relevance in that insulin may be implicated in the cause of a death. However, multi-stage sample preparation methodologies not commonly employed in toxicology labs, instability in standard lab ware or specimen containers, and the necessity to analytically differentiate the nearly identical pharmaceutical analogs has remained a challenge for the forensic community. Consequently, the determination of insulin in postmortem cases is not routinely performed.

Objectives: The goal of this project is to develop a straightforward, robust, and forensically valid approach for the differentiation and quantitation of human insulin as well as five pharmaceutical analogs, including insulin Glargine (Lantus®), Glulisine (Apidra®), Lispro (Humalog®), Aspart (NovoLog®), Human (Humulin®), and Detemir (Levemir®) in post-mortem vitreous humor.

Methods: Robotic immunoaffinity extraction was performed on the Agilent AssayMap Bravo automation system using 200 µL of vitreous humor. LC-MS/MS analysis on an Agilent 6495 triple quadrupole mass spectrometer coupled with a 1290 series UHPLC measured insulin β-chains to unequivocally differentiate each analog. Chromatographic separation was performed using an Agilent RRHD 300Å SB-C18 1.8 µm 2.1 x 50mm analytical column with a stepwise separation at 0.4 mL/min over 9 minutes. Mobile phases consisted of water with 0.2% formic acid and acetonitrile with 0.2% formic acid. Method validation was performed in accordance with SWGTOX guidelines.

Results: All analogs performed within the criteria for acceptable performance. Validation evaluated linear range (500 pg/mL – 25,000 pg/mL), limit of quantitation (500 pg/mL), limit of detection (500 pg/mL for insulin Detemir and 125 pg/mL for all other analytes), accuracy and precision (total difference/CV < 20%), interference, carryover, and stability. To date, this method has been used to test 38 vitreous fluid specimens submitted by medical examiners and other death investigators. Evaluation of the data shows that 29% (n=11) tested positive (n=6 for Lispro; n=3 for Aspart; n=2 for Human Insulin).

Conclusion/Discussion: Overall, this workflow has been successfully developed, validated, and applied to authentic forensic samples. Detailed case histories from several cases demonstrate the utility and accuracy of the method. Ongoing efforts aim to optimize and expand the scope of the assay. This effort has focused on improvements to the extraction so that it can be applied to tissue samples that may contain injection marks as well as post-mortem blood. In addition, C-peptide, as well as Insulin Degludec (Tresiba®), are being added to the scope of the assay.
S16: Improving Scope and Turnaround Times in Postmortem Casework with a Consolidated Methodology Approach

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Background/Introduction: Systematic toxicological approaches that employ both ideology changes and improvements in instrumentation and sample extraction allow for increased efficiency through lower sensitivities, higher specificity and minimizes resources used. Traditional methods utilizing GC-MS typically require iterations of testing, which hinder productivity and turnaround times, particularly for polypharmacy cases frequently seen in modern postmortem toxicology. This two-part presentation aims to discuss the method development and validation of a LC-MS/MS analytical method and to demonstrate how the transition from classic GC-MS methods enhances postmortem toxicology testing and accurate cause and manner of death decisions.

Objectives: The objective of this project was to develop a LC-MS/MS method that consolidates the scope of seven legacy methods for better sensitivity, higher throughout, quantitation of drugs of abuse with minimal sample consumption, and incorporation of smart automated processing for improved quality assurance.

Methods: One hundred microlitres of blood or urine were rapidly extracted using a simple acetonitrile protein crash and subsequent in-vial filtration (Thomson Instrument Company, New Jersey, US) with nitrogen dry-down and starting conditions reconstitution. The LC-MS/MS system was a Sciex Nexera X2 LC-30 with a Sciex QTRAP 6500+ mass spectrometer utilizing an Ion Drive™ Turbo V electrospray ionization (ESI) source in positive mode. Chromatography utilized a gradient on a Kinetex Phenyl Hexyl 100 Å LC Column (100 x 4.6 mm, 2.6 μm) column (Phenomenex, California, US). 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 (LOD); Limits of Quantitation; Matrix Effects; Processed Sample Stability; and Linearity experiments. To demonstrate applicability, the described method was applied to previous proficiency test samples and the blood samples of the last 12 months of authentic casework analyses were compared to the scope and limits of detection from the laboratory’s legacy methods.

Results: This analytical method incorporates 55 analytes and the customized query facilitates rapid and consistent application of acceptability criteria for data processing and review. This method analyzed 1389 samples (858 blood and 531 urine). There were 2551 analytes (average of 3.0 per sample) and 1938 analytes (average of 3.6 per sample) detected in blood and urine, respectively. Of the top 5 in blood being methamphetamine, amphetamine, morphine, benzoylecgonine, and cocaine, with similar findings in urine. Due to the narrower scope and decreased sensitivity of the seven legacy GC-MS methods, at least 41% of results would have been missed if indeed all seven GC-MS methods were employed for each sample which was atypical. Furthermore, 11% of results were not within the previous scope of our analytical methods.

Conclusion/Discussion: Historically, the San Francisco Office of Chief Medical Examiner relied heavily on a GC-MS testing regime, comprised of individual drug-class confirmation and quantitation assays, typically requiring iterations and prioritization of testing given insufficient sample volumes. The analytical method presented here significantly increases the efficiency and throughput for postmortem casework. Cases containing any of the top 5 analytes would have typically required three distinct GC-MS assays, 3 mL of blood, and upwards of 30 hours of active staff time. The described LC-MS/MS analytical approach mitigates the need to perform multiple assays, utilizes only 0.1 mL of sample, and reduces the extraction time from 16 hours to 3.5 hours. Incorporation of 10 additional analytes, including prescription medication and opioids, allows for a more comprehensive testing regime to better inform pathologists for cause of death determinations. This method promotes consistency and standardization in quality assurance while increasing efficiency in case management and decreasing turnaround times, pivotal in modern postmortem toxicology.

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Background/Introduction: Carbon monoxide (CO) poisoning presents an interesting challenge for post-mortem toxicology laboratories. The co-oximeter, a device based on spectrophotometric principles and developed for % carboxyhemoglobin (COHb) determination, became extremely popular in post-mortem laboratories; it provides simple and quick sample preparation along with instant results while removing the need for any deep understanding of the principles underlying its function. One brand of co-oximeter, Instrumentation Laboratory (IL), became particularly popular. A recent survey of post-mortem laboratories (n=17) indicated a majority currently use or formerly used IL co-oximeters for their CO testing. Recently, the IL brand has been discontinued and less technical support is offered. Laboratories, therefore, need to find alternative solutions. One particularly appealing alternative included spectrophotometric methods which would boast similarly simple and quick sample preparation procedure while at the same time offering enough precision and accuracy for post-mortem work. A major hurdle for laboratories is the selection of the appropriate spectrophotometric method to determine %COHb, as a variety of procedures have been published over the years.

Objective: The objective of this work was to compare four spectrophotometric methods for determining %COHb using bias, precision, sensitivity, ease of analysis and the effect of potential interferences commonly observed in postmortem samples.

Methods: The four methods were chosen based on literature findings and/or the appropriateness for postmortem testing. These methods are based on (A) relating %COHb to the ratio of COHb and Hemoglobin (Hb) absorbance of a reduced sample, (B) multicomponent analysis of all hemoglobin species, (C) multicomponent analysis of a reduced sample and (D) derivative spectroscopy. All samples were analyzed with the same conditions using an Agilent CARY 60 Spectrophotometer with instrument control and data processing performed using Cary WinUV Software. Samples were prepared according to the specifications given in published papers that effectively used the method in question for spectrophotometric analysis. Postmortem samples that previously tested positive for CO were utilized for analysis in addition to blood samples that were prepared by bubbling CO into human blank blood.

Results: Methods B and C boasted similar within-run and between run-precision (<5%) with methods A and D being notably less precise at lower concentrations but still within lab standards for %CV (<20%). In terms of bias, all methods produced results with acceptably low bias (<20%) with methods B and D being below 5%. In terms of dynamic range, all methods were linear \( r^2 > 0.99 \) at low range (~ 3 % COHb) up to 100% COHb except for method D, which had notable discrepancies at low %COHB (<10%) and extremely high %COHB (>70%). There was variation across methods, but general agreement within 10% COHb for the majority of post-mortem samples analyzed. There were a few slight trends, such as method B producing lower values and method D producing higher %COHb than expected. Samples exposed to excessive heat showed no major trends across methods. Decomposed samples, on the other hand, produced more accurate results using method B, with methods C and D having moderately over-calculated %COHB values (47-53% error) and method A's over-calculations appearing more extreme (~70% error). In terms of ease of analysis (sample preparation time, data analysis etc.), method B is by far the simplest with methods C and D being the most involved.

Conclusions/Discussion: While the methods performed similarly in terms of typical validation requirements, the ability to more effectively deal with decomposed samples and simple sample preparation made method B (multicomponent analysis) most suitable for post-mortem testing.

Keywords: Carboxyhemoglobin, Post-mortem, Hemoglobin, Co-Oximetry, Spectrophotometry, Carbon Monoxide
**S18: Rapid Method by LC-QTOF-MSE for the Addition of Prevalent Synthetic Cannabinoids in a Forensic Toxicology Laboratory**

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**Background/Introduction:** Synthetic cannabinoids (SCs) are one of the largest groups of novel psychoactive substances being monitored by the Drug Enforcement Administration (DEA) (DEA Emerging Threat Report, 2018). It was reported in 2016, that over 240 SCs have been identified worldwide (Ambroziak and Adamowicz, 2018). The abuse of these compounds poses a serious threat to the public health, health care systems, and both forensic and clinical toxicology laboratories. Similar to what has been reported nationally by several organizations, Washington DC has experienced spikes in emergency calls due to SC usage. The emergence of new synthetic cannabinoids requires both a prompt response and a selective method that allows for the detection of prevalent SCs.

**Objectives:** Create a rapid and selective analytical screening method for multigenerational SCs in blood that would enable a forensic toxicology laboratory to better respond to a public health crisis.

**Methods:** Extraction optimization was conducted using liquid-liquid extraction on drug free antemortem and postmortem blood fortified with the analytes of interest. The parameters evaluated were extraction solvent ratio, buffer pH, as well as the optimal volume for both sample aliquot and reconstitution. Based on optimization results, sample preparation was conducted via a liquid-liquid extraction with 1.0 mL blood extracted into 4 mL 80:20 hexane/ethyl acetate and 1 mL 0.13M sodium borate buffer (pH 9.3). Samples were then mixed for 10 minutes on the Orbital Mixer, sonicated for 5 seconds to remove emulsions, and centrifuged at 3,000 rpm for 5 minutes. The upper organic layer was immediately transferred to clean conical tubes and evaporated at 40 °C under nitrogen. Following evaporation, samples were reconstituted in 50 μL of 50:50 mobile phases, vortex mixed, and transferred to labeled autosampler vials for analysis.

Analyses were carried out using a Waters Acquity Ultra pressure liquid chromatograph (UPLC) paired with a Waters Xevo G2 mass spectrometry-quadrupole time of flight detector. Reference standards purchased from Cayman Chemical (Ann Arbor, MI, USA) were utilized for chromatographic optimization to evaluate the following parameters: analytical column, injection volume, gradient and source optimization, reconstitution volume, and chromatographic separation. Each parameter was evaluated based on retention time, fragment ions, and detector counts. Per the results of the optimization experiments, separation was performed on a CORTECS UPLC C18 (1.6 µm, 2.1 mm X 100 mm) analytical column using a 5 mM ammonium formate in water (A): 0.1% formic acid in acetonitrile (B) with a flow rate of 0.5 mL/min and a run time of 11 minutes.

Data were processed using Waters UNIFI Scientific Information Software in data independent acquisition mode. The processing method consisted of a compound library of 36 compounds and 6 internal standards, each having an expected retention time, expected neutral mass, and expected fragment ions. The criteria for identification was mass error <2 ppm, RT error <0.35 min, the identification of the expected fragment ions, and peak areas >200 counts.

**Results:** A compound database library was created for 36 prevalent multi-generational synthetic cannabinoids in the District of Columbia. The method allowed for the simultaneous detection and identification of all 36 analytes. With the discovery of new compounds, this method allows for the rapid analyte addition of new analytes and identification based on accurate mass, retention time, and fragment ions.

**Discussion/Conclusion:** An LC-QTOF method was developed for the simultaneous detection of 36 synthetic cannabinoids in antemortem and postmortem blood. This method utilizes a library database for rapid screening of synthetic cannabinoids and allows for library expansion when new SCs emerge, providing a sensitive and specific analytical screening method to meet public health needs.

**Keywords:** synthetic cannabinoids, blood, LC-QTOF-MS, optimization, library
Background/Introduction: The misuse of designer benzodiazepines, as an alternative to prescription benzodiazepines and for drug-facilitated sexual assaults, has emerged as a growing threat, due in part to the ease of purchasing these drugs on the internet. Causing concern for safety, is the lack of dosage information resulting in users self-medicating, often leading to unintended overdoses, coma, or death at higher doses.

Objectives: With limited published data regarding the quantification of designer benzodiazepines in forensic cases, the main objective was to develop and validate a method for the determination of thirteen designer benzodiazepines in postmortem blood, to add to the in-house method that already included a limited number of common designer benzodiazepines.

Methods: The developed method consisted of analysis for 3-hydroxyphenazepam, clobazam, clonazolam, delorazepam, deschloroetizolam, diclazepam, flualprazolam, flubromazepam, flubromazolam, flunitrazolam, meclonazepam, nifoxipam, and pyrazolam in 0.5 mL postmortem blood using LC-MS/MS. The analytes were treated with solid phase extraction before undergoing separation on a column and analyzed on the mass spectrometer in electrospray positive mode using multiple-reaction monitoring. The parameters tested for method validation consisted of linearity, bias, precision, limit of quantitation (LOQ), limit of detection (LOD), matrix effect, carryover, stability, interference, and dilution integrity. The validated method was then applied to blood specimens from the New York City Office of Chief Medical Examiner.

Results: The linear range of the calibration curve was 1-200 ng/mL, and up to 500 ng/mL for 3-hydroxyphenazepam, clobazam, flubromazepam, and pyrazolam. The limits of detection and quantitation were 0.5 ng/mL (signal/noise (S/N)>3) and 1 ng/mL, respectively. The calculated bias, intra-day imprecision, relative standard deviation (RSD), and inter-day imprecision RSD were ±12%, 3-20%, and 4-21%, respectively. Matrix effects ranged from -52% to 33% with RSD values ranging from 3-20%, indicating consistent effects throughout multiple sources. Recovery ranged from 35-90%, where only two compounds were below 50%. Carryover was not present in samples with concentrations up to 1000 ng/mL. Dilution integrity was maintained for all compounds, except diclazepam in the 1:5 dilution and nifoxipam in the 1:2 dilution. Interferences caused by endogenous and exogenous compounds were not observed regarding the identification of the target compounds. Upon storage in the autosampler for stability studies, nifoxipam and flubromazepam displayed deterioration, while clonazolam, deschloroetizolam, diclazepam, flualprazolam, and flunitrazolam quantified greater than 25% of the original concentration after 24 h. Of the 33 blood specimens that were re-analyzed using this method, five samples tested positive for designer benzodiazepines consisting of clonazolam, delorazepam, diclazepam, flualprazolam, and flubromazolam, with concentrations from 0.93 to 68.91 ng/mL.

Conclusion/Discussion: The validated method consists of the simultaneous determination of 13 designer benzodiazepines in blood using solid phase extraction (SPE) and a 13.5-minute analysis on the LC-MS/MS, proving to be simple, reproducible, sensitive, and robust.

Keywords: Designer Benzodiazepines, LC-MS/MS, Blood
S20: 5F-MDMB-PINACA and 5F-MDMB-PICA Metabolite Identification and Cannabinoid Receptor Activity

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Introduction: According to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), there were 179 synthetic cannabinoids reported as of 2017. In the United States, there were 24,501 identifications of synthetic cannabinoids reported to the National Forensic Laboratory Information System (NFLIS) in 2017. The synthetic cannabinoid, 5F-MDMB-PINACA or 5F-ADB, accounted for 28% of those identifications. The synthetic cannabinoid, 5F-MDMB-PICA, is structurally similar to 5F-MDMB-PINACA with an indole group replacing the indazole. While very little is known about 5F-MDMB-PICA, its pentylfluoro side chain like that of 5F-MDMB-PINACA, indicates high potency at the cannabinoid 1 (CB1) receptor. Limited data exist from in vivo or in vitro metabolic studies of either of these synthetic cannabinoids, so potential metabolites to identify use may be missed.

Objectives: To investigate metabolism of both 5F-MDMB-PINACA and 5F-MDMB-PICA utilizing human hepatocyte incubations to identify in vitro metabolites. To examine authentic case specimens that involved these synthetic cannabinoids in order to verify metabolites identified by hepatocyte incubations. Also, to identify the potency and efficacy of 5F-MDMB-PINACA and 5F-MDMB-PICA by examining activity at the CB1 receptor.

Methods: 5F-MDMB-PINACA and 5F-MDMB-PICA were incubated with pool human hepatocytes (20-donor). Ice cold acetonitrile was used to end incubations after 1, 3, and 5 h. An Agilent 1290 infinity ultra-high performance liquid chromatography system coupled with an Agilent 6550 iFunnel quadrupole time-of-flight (QTOF) mass spectrometer equipped with a Dual Agilent Jet Stream electrospray ionization source was used for the analysis. Data from hepatocyte incubations and urine samples were processed using a Personal Compound Database and Library (PCDL) generated in-house using MassHunter PCDL that included common and expected phase I and phase II metabolites. Postmortem (n=3) and antemortem (n=1) urine samples were analyzed with and without hydrolysis.

Analysis of receptor activation was carried out on aequozen recombinant CHO-K1 cell lines expressing the human CB1 receptor using a coelenterazine based luminescence assay and a Spark 10M plate reader. Dose response curves were prepared in triplicate and compared to those of JWH-018.

Results: Biotransformations found in this study included phase I transformations (amide hydrolysis, carboxylation, dehydrogenation, ester hydrolysis, N-dealkylation, hydroxylation, oxidative defluorination, oxidative defluorination to pentanoic acid, propionic acid formation at the indole/indazole side chain) and phase II transformations (glucuronidation). A total of 21 5F-MDMB-PINACA metabolites (A1 to A21) were identified with 3 compounds unique to urine specimens. From hepatocyte incubations and urine samples, 22 metabolites (B1 to B22) were identified for 5F-MDMB-PICA in this study. Phase II metabolites (glucuronides) were identified in 5F-MDMB-PINACA (n=5) and 5F-MDMB-PICA (n=3). Receptor activation studies concluded that 5F-MDMB-PINACA and for 5F-MDMB-PICA were equally potent as JWH-018 at the CB1 receptor.

Discussion/Conclusion: For both compounds, ester hydrolysis and ester hydrolysis in combination with oxidative defluorination were the most abundant metabolites produced in vitro. At least one of these biotransformations were present in each of the case samples presented and are in agreement with previous literature. Both 5F-MDMB-PICA and 5F-MDMB-PINACA were found to be active at the CB1 receptor with potency similar to JWH-018.

Keywords: 5F-ADB, 5F-MDMB-PICA, Hepatocyte Metabolism, Synthetic Cannabinoids, CB1 activity
S21: Quantitation of Tianeptine and its Metabolite in Blood and Urine by LC-MS/MS

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Background/Introduction: Tianeptine (Stablon, Coaxil, or Tatnol) has been prescribed throughout countries in Europe, Asia, and South America as an antidepressant since the late 1980’s. However, within the United States, its use has not been approved by the U.S. Food and Drug Administration, but it can be readily purchased online as a dietary supplement. While structurally similar to tricyclic antidepressants (TCAs), tianeptine has a unique mechanism of action by enhancing the reuptake of serotonin. Studies have also shown that tianeptine is a mixed mu- and delta-opioid receptor agonist that is less potent than morphine. Its active metabolite, MC5, is formed from extensive b-oxidation and has a similar potency to its parent compound at mu-opioid receptors but little activity at delta-opioid receptors. These characteristics have led to recent increased misuse and abuse across the United States.

Objectives: The objective of this project was to develop and validate a quantitative method for the analysis of tianeptine and its active metabolite, MC5, in blood and urine specimens using liquid chromatography tandem mass spectrometry (UPLC-MS/MS). Case samples, tentatively identified to contain tianeptine, were then analyzed for quantitative confirmation using the validated method.

Methods: Method validation was conducted according to the Scientific Working Group for Toxicology (SWGTOX) guidelines. Parameters assessed included linearity, limit of detection (LOD), limit of quantitation (LOQ), extraction recovery, precision, bias, dilution integrity, stability at 3 temperatures (-20°C, 4°C, 23°C), interferences, and ionization suppression/enhancement. To 200 µL aliquots of matrix, 25 µL of deuterated internal standard for each analyte was added followed by 3 mL of phosphate buffer (pH 6). Samples were then subjected to a solid phase extraction optimized using 130 mg Clean Screen® DAU extraction columns (UCT). Following elution and evaporation, samples were reconstituted in initial mobile phase conditions for analysis using a Waters Acquity U Series UPLC® coupled to a Waters Xevo® TQD mass spectrometer. Chromatographic separation was achieved on an Acquity UPLC® BEH C18 column (2.1 x 100 mm, 1.7 µm) with 5 Mm ammonium formate pH 4 and 0.1% formic acid in methanol using gradient elution and a flow rate of 0.45 mL/min.

Results: All validation parameters were deemed acceptable based on SWGTOX guidelines. Baseline resolution was achieved for both analytes in under 4 minutes. Linearity was determined to range from 25-1000 ng/mL using 6 points of calibration. LOD and LOQ were set to 25 ng/mL. Extraction recovery was 60% for both analytes. Inter- and intra-run precision ranged from 1.6 to 8.6%. Bias was calculated to be ≤ 6% for both analytes. Dilution integrity was evaluated at 1:2, 1:10, and 1:40 and had no significant impact on accuracy of results. No interferences were observed from the matrices tested or from commonly encountered drugs of abuse, and no carryover was detected in blank samples run immediately after injecting the highest calibrator (1,000 ng/mL). Both analytes were stable in blood and urine at all temperatures for 30 days. Six blood and three urine samples were analyzed using this method. Concentrations ranged from 620-10,000 ng/mL with a median of 1,000 ng/mL for tianeptine, and 1,300-12,000 ng/mL with a median of 1,900 ng/mL for MC5 in blood. In urine, concentrations ranged from 2,700-28,000 ng/mL with a median concentration of 12,000 ng/mL for tianeptine while all cases were >40,000 ng/mL for MC5.

Conclusion/Discussion: The method was optimized and fully validated for the quantitation of tianeptine and its active metabolite MC5 in human blood and urine. To our knowledge, this is the first method that quantitates both tianeptine and MC5 in these two matrices using UPLC-MS/MS technology in forensic casework. The method was successfully applied to the analysis of 9 samples from 5 cases and generated quantitative results which will aid in the interpretation of future cases.
S22: Method Development and Validation of U-Type Opioid (Utopioid) Series Compounds and their Identification in Authentic Case Samples

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Background/Introduction: U-Type Opioid (“Utopioid”) series compounds were originally developed in the 1970s by the Upjohn pharmaceutical company as opioid analgesics. Despite their intended medical use, they were never studied in humans. Beginning in 2015 however, compounds from this series (e.g. U-47700) began to appear in forensic casework. Since 2015, additional compounds from the series have proliferated, creating analytical challenges due to the occurrence of several isomers, especially ring substitution, and their chemically similar nature. Differentiation of isomeric species is unachievable solely by mass spectrometry techniques due to same chemical formulae and their very similar fragmentation patterns. This necessitates chromatographic separation for definitive identification of these compounds. On the current illicit market, there are two specific sets of utopioid isomers: U-48800/U-51754 and U-49900/Propyl-U-47700/Isopropyl U-47700 that exemplify this challenge.

Objectives: The purpose of this presentation is to describe an analytical method capable of chromatographically resolving the isomeric species within the utopioid class of compounds. In addition, we discuss the concentrations of the drugs found in authentic biological specimens (blood and/or urine).

Methods: A method was developed and optimized using a Waters Xevo TQ-S Micro tandem mass spectrometer coupled with a Waters Acquity UPLC® (Milford, MA). Chromatographic separation was achieved using a gradient elution on a Waters Cortecs® UPLC® C18 column (2.1 mm x 100 mm, 1.6 um), heated to 60°C with a flow rate of 0.3 mL/min. The mobile phases used for analysis were 5 mM ammonium formate (pH=3, MPA) and 0.1% formic acid in acetonitrile (MPB).

The following utopioid analytes were included in this method: 3,4-methylenedioxy-U-47700, U-50488, 3,4-ethylenedioxy-U-51754, 3,4-ethylenedioxy-U-47700, U-48800, U-47700, U-49900, propyl-U-47700, N-desmethyl-U-47700, U-69593, U-51754, N,N-didesmethyl-U-47700, 4-phenyl-U-51754, isopropyl-U-47700 and U-47931E (bromadoline).

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

Results: The method was validated according to ASB guidelines, evaluating the following criteria: bias, intra- and inter-assay precision, linearity, limit of detection (LOD), limit of quantitation (LOQ), matrix and commonly encountered interferences. All isomers were successfully resolved chromatographically.

U-48800 cases (n=10) were detected by NMS Labs (Willow Grove, PA) from January to December 2018, submitted from Florida (n=4), Pennsylvania (n=3), Ohio (n=1), Minnesota (n=1) and South Carolina (n=1). Case histories were provided with the corresponding concentrations for two cases. Case 1: a 46 year-old female was found in the supine position on the bedroom floor with heroin packets stamped with a blue skull wearing a hat and the words “Stranger Danger”. The concentration of U-48800 was 5.3 ng/mL, in addition to other opioids and common drugs of abuse found. Case 2: a 41-year-old male was found deceased in the bathroom of a hotel room. Drug paraphernalia was found as well, with the heroin packet having the same stamp and markings as Case 1. The concentration of U-48800 was <1 ng/mL, in addition to fentanyl and cocaine present.

Seven U-49900 cases were detected by NMS Labs from April 2017 to January 2018 from the following states: Illinois (n=3), Georgia (n=1), Missouri (n=1) and Pennsylvania (n=1). All cases were male subjects and U-49900 was found in combination with novel opioids.

Conclusion/Discussion: The utopioid series creates analytical challenges for forensic analysis, specifically due to isomeric pairs. The ten cases testing positive for U-48800 in this study screened positive for U-51754 during the initial analytical testing. Without definitive separation of the utopioids, U-48800 would not have been confirmed in these cases. Forensic laboratories should be aware of the utopioid series and develop methods for their identification and confirmation.

Keywords: U-series, Postmortem, LC-MS/MS
S23: Evaluation of Synthetic Cannabinoid Metabolites in Human Blood in the Absence of Parent Compounds

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Background/Introduction: Synthetic cannabinoids present unique challenges for detection in toxicological samples compared to other classes of novel psychoactive substances (NPS). Historically, synthetic cannabinoid testing has consisted of targeted assays for parent compounds in blood and metabolites in the urine; however, few laboratories evaluate the presence of metabolites in blood. Following internal1 and external2 reports of metabolites present in blood in the absence of parent compounds, our laboratory re-evaluated our analytical approach to assess the stability of synthetic cannabinoids in blood under various storage conditions and the prevalence of metabolites only in authentic forensic toxicology cases.

Objectives: The objective of this study was to determine the stability of 5F-ADB (5F-MDMB-PINACA), FUB-AMB (MMB-FUBINACA), ADB-FUBINACA, and 5F-MDMB-PICA in human whole blood. 5F-ADB, FUB-AMB, and 5F-MDMB-PICA all have terminal methyl esters, a moiety hypothesized to be more susceptible to breakdown or conversion. These four analytes represented the most commonly encountered synthetic cannabinoids in the United States in 2018 and early 2019. Additionally, the prevalence of these parent compounds and/or their metabolites was investigated.

Methods: Preserved human whole blood was fortified with 5F-ADB, FUB-AMB, ADB-FUBINACA, and 5F-MDMB-PICA at 10 ng/mL. Blood aliquots were stored at room temperature (n=3), refrigerated (n=3), and frozen (n=3) for 1, 2, 3, 7, 14, 21, and 35 days (total n=27). At the given intervals, blood samples were prepared for analysis by liquid-liquid extraction. Blood samples (0.5 mL) were acidified with 5% phosphoric acid in water (1 mL) and extracted into a mixture of hexane, ethyl acetate, and methyl tert-butyl ether (3 mL, 80:10:10 v:v). Extracts were analyzed via a Sciex TripleTOF® 5600+ quadrupole time-of-flight mass spectrometer coupled with a Shimadzu Nexera XR ultra high performance liquid chromatograph (LC-QTOF) using SWATH® non-targeted acquisition. The stability of each analyte was determined by monitoring change in peak area ratio over time. In addition, blood extracts were obtained from NMS Labs for analysis by LC-QTOF using the same method used for monitoring stability.

Results: FUB-AMB was found to be highly unstable, with no parent compound detectable after only 1 day when stored at room temperature and after only 3 days when stored refrigerated. 5F-ADB was also found to be highly unstable, with noticeable loss in parent compound (up to 90%) after 7 days when stored at room temperature or refrigerated. 5F-MDMB-PICA was found to be the most stable of the methyl esters; however, more than 75% of the parent compound was lost after 30 days. The degradation of all three compounds (5F-ADB, FUB-AMB, and 5F-MDMB-PICA) corresponded to an increase in their respective butanoic acid metabolites: 5F-ADB 3,3-dimethylbutanoic acid, FUB-AMB 3-methylbutanoic acid, and 5F-MDMB-PICA 3,3-dimethylbutanoic acid. This indicates that the presence of these metabolites in blood samples may be due, at least in part, to instability. Since March 2018, 193 toxicology cases were positive for 5F-ADB 3,3-dimethylbutanoic acid (n=114) and FUB-AMB 3-methylbutanoic acid (n=102). Of these cases, 83 blood samples (43%) were positive for synthetic cannabinoid metabolite(s) in the absence of the parent compound. FUB-AMB 3-methylbutanoic acid was detected with the highest frequency without FUB-AMB (n=53), consistent with the results of the stability study. 5F-ADB 3,3-dimethylbutanoic acid was detected without 5F-ADB in 43 blood samples. To date, we have not identified 5F-MDMB-PICA 3,3-dimethylbutanoic acid in the absence of 5F-MDMB-PICA in blood samples.

Conclusion/Discussion: 5F-ADB, FUB-AMB, and 5F-MDMB-PICA were found to be unstable in preserved human whole blood, based on storage conditions. These analytes were found to degrade to their respective butanoic acid metabolites. Laboratories should analyze for synthetic cannabinoid metabolites in blood in addition to the parent compounds, as these findings are toxicologically significant.

References:
Background/Introduction: In comprehensive toxicological drug screening, robust identification criteria is necessary to ensure that all drugs of abuse, therapeutics, poisons and novel psychoactive substances are correctly identified. It is pivotal for any laboratory routinely investigating increasing numbers of deaths, drug facilitated crimes (DFC) and drug impaired driving (DUID) casework to efficiently process results and thus, to significantly mitigate the amount of resources required to produce high quality reporting in medico-legal settings. Over 700 compounds, including anesthetics, analgesics, anticonvulsants, antidepressants, antihistamines, antipsychotics, barbiturates, benzodiazepines, cannabinoids, cathinone, cocaine and metabolites, fentanyl, hallucinogens, opioids, phenylethylamines, new psychoactive substances (NPS), and nonsteroidal anti-inflammatory drugs (NSAID), were analyzed by ultra-high-performance liquid chromatography (UHPLC) coupled with a quadrupole-time of flight mass spectrometer (QTOF/MS) system, a high-resolution mass spectrometry (HRMS) technology that utilizes sequential window acquisition of all theoretical fragment-ion spectra (SWATH). Variations in criteria for mass error, retention time, percentage of isotope ratio difference, and library hit scoring parameters along with percentage allocations of these parameters were optimized to determine a threshold for combined weight scores. The scores within individual parameters also provided further insight to the identification confidence. The system was designed as an automated workflow with pre-set criteria for inclusion, consideration and exclusion.

Objectives: To optimize and validate the identification criteria system that obtained the highest efficiencies allowing for the accurate screening of casework by HRMS.

Methods: A simple, nonspecific sample preparation technique was employed. The UHPLC system consisted of an ExionLC coupled with a SCIEX LC-QTOF/MS X500R system (AB Sciex LLC, Framingham, MA, USA). Separation was performed using a Phenomenex Biphenyl (50 x 4.6 mm, 2.6 µm) at a flow rate of 1.2 mL/min. The 700 compounds were prepared using certified reference materials purchased from Cerilliant (Round Rock, TX, USA), Cayman (Ann Arbor, MI, USA) and Lipomed (Cambridge, MA, USA). Limits of Scope (LOS) for each compound were determined based on satisfactory signal to noise (S/N) ratios and integration review. LOS mixes were then prepared and extracted in blood in triplicate over 3 days as recommended in the SWGTOX guidelines. Additionally, settings were varied within each of the four identification criteria parameters (mass error, retention time, percentage of isotope ratio difference, and library hit scoring parameters) along with percentage allocations of these parameters were optimized to determine a threshold for combined weight scores. The scores within individual parameters also provided further insight to the identification confidence. The system was designed as an automated workflow with pre-set criteria for inclusion, consideration and exclusion.

Results: The most favorable system was selected for the greatest number of compounds. Customized rules were then determined for compounds not exhibiting targeted efficiency with the generalized criteria. The efficiency, sensitivity, specificity and positive predictive value (PPV) of the software’s native traffic light system was then determined. Finally, an applicability validation study was performed using authentic case specimens and proficiency test samples providing further feedback regarding accurate identification of analytes.

Conclusion/Discussion: The identification criteria determined was optimized for efficiency in the setting of the sample matrix. This subsequently allowed for the traffic light system and a combined weight score objectively that mitigates false negative results in an automated workflow solution for high throughput forensic toxicology laboratories. The described HRMS method provides an efficient, comprehensive, and accurate toxicological testing regime for immediate reporting in postmortem, DUID and DFC casework received by the San Francisco Office of the Chief Medical Examiner.
S25: Structural Identification of Metabolites of Synthetic Cannabinoids JWH-018, AM-2201, THJ-018, THJ-2201 and 5F-AKB-48 Using In-house Synthesized Standards, Hepatocytes & LC-QTOF-MS

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Background/Introduction: Synthetic cannabinoids are quickly metabolized and often, only the metabolites can be identified in urine. Therefore, the elucidation of metabolites and the production of metabolite reference materials is especially important.

These metabolites are mainly identified through metabolism studies using either in vitro models such as liver microsomes, cell lines or hepatocytes, or authentic urine samples. Metabolites are identified using high resolution LC-MS. While high resolution LC-MS is a powerful analytical technique, it is limited in its ability to distinguish structural analogs, such as hydroxyl group position.

Objectives: The aim of this study was to produce or obtain reference materials for synthetic cannabinoid metabolites and identify the exact structure of the metabolites produced by hepatocytes with a special focus on those that cannot be unambiguously identified by their MSMS-spectra.

Methods: Thirty-one metabolites of JWH-018, AM-2201, THJ-018, THJ-2201 and 5F-AKB-48 were synthesized in-house. They were characterized by LC-MS and NMR. Nine metabolites from the same parent analytes were obtained from commercial sources.

For JWH-018, the 2-, 4- and 5-OH-pentyl, 5-, 6- and 7-OH-indole, 4-OH-naphthyl, 2,3- and 4,5-diOH-pentyl, as well as the pentanoic acid were available. For AM-2201 the 2-, 3- and 4-OH-pentyl, 2,3- and 4,5-diOH-pentyl were available. For THJ-018 the 2-, 3-, 4- and 5-OH-pentyl, 2,3- and 4,5-diOH-pentyl were available. For THJ-2201 the 2-, 3- and 4-OH-pentyl were available. For 5F-AKB48, 2-, 3- and 4-OH-pentyl, as well as 4eq- and 4-ax-OH-adamantyl were available. Metabolites from 5-fluorinated-analogs were also compared to potential non-fluorinated metabolites. As such, for AKB48, N-dealkylation alone and in combination with 4-ax-, 4-eq- and tert-OH-adamantyl, 5-OH-pentyl alone and in combination with 4-ax- and 4-eq-OH-adamantyl, pentanoic acid alone and in combination with 4eq-, 4ax- or tert-OH-adamantyl, were also included.

The parent drugs were incubated with cryopreserved hepatocytes (5 µM, 1 million cells/ml, 5 h). After stopping the reaction with acetonitrile, the supernatants were analyzed by LC-QTOF-MS (Agilent 6550) using mobile phases consisting of ammonium formate and acetonitrile on an HSS T3 column (Waters).

Hepatocyte metabolites were matched with reference materials based on retention time, mass, and when available, MSMS-spectra.

Results: After incubation with the non-fluorinated cannabinoids JWH-018 and THJ-018, 4-OH-pentyl metabolites were observed. Similarly, after incubation with the fluorinated analogs AM-2201 and THJ-2201, 5-OH-pentyl metabolites were observed. In addition, for AM-2201, the pentanoic acid metabolite was observed.

For 5F-AKB48, the major monohydroxylated metabolite was 5-OH-pentyl, but 4eq-OH-adamantyl was also observed together with the corresponding dihydroxylated metabolite (5-OH-pentyl,4eq-OH-admantyl), as well as with the pentanoic acid metabolite. In addition, a metabolite matching the reference materials of both pentanoic acid in combination with 4eq-OH-adamantyl and pentanoic acid in combination with tert-OH-adamantyl was observed.

Conclusion/Discussion: When analyzing a series of structural analogs, chromatographic separation is particularly important. In this study, most analogs were well separated but still, one of the metabolites had two co-eluting structural analogs. As reference material for both 5F-AKB48 pentanoic acid,tert-OH-adamantyl and 5F-AKB48 pentanoic acid, 4eq-OH-adamantyl were available it became apparent that they could not be differentiated. That said, 4eq-OH-adamantyl seem to be the preferred isomer for other metabolites, which were separated from their tert-OH-adamantyl counterparts.

This study provides new insights into the preferred sites of metabolism of cannabinoid analogs that could be used in future metabolism studies, as well as for the synthesis of reference materials. This is especially true for 5F-AKB48, where 4eq-OH-adamantyl appears to be a preferred site of metabolism.
S26: 4F-MDMB-BINACA: An Emergent Synthetic Cannabinoid Implicated in Forensic Toxicology Casework
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Background/Introduction: As the number of emergent opioids and fentanyl analogues has been decreasing, the number of new synthetic cannabinoids has increased again within the last year. 5F-MDMB-PICA and 4F-MDMB-BINACA have recently emerged and become more prevalent as the positivity of 5F-ADB and FUB-AMB have declined. Of concern to forensic toxicologists is the prevalence of synthetic cannabinoids in medicolegal death investigations (MDI) and driving under the influence of drugs (DUID) investigations. Synthetic cannabinoids present analytical challenges as a result of their complex and diverse chemistries, specifically issues involving recovery of parent compounds and metabolites from biological samples, unknown metabolic profiles, and need for high sensitivity. This presentation focuses on 4F-MDMB-BINACA, one of the most prevalent synthetic cannabinoids in use in the United States in 2019 and an analyte that may not be widely incorporated into forensic toxicology testing protocols.

Objectives: This study sought to characterize the emergence and prevalence of 4F-MDMB-BINACA in forensic toxicology casework. In addition, the in vivo metabolism of 4F-MDMB-BINACA was investigated by analysis of authentic casework samples to identify metabolites for incorporation into blood and urine testing protocols.

Methods: Extracts of blood and urine samples, correlating to cases of suspected synthetic cannabinoid use, were obtained from NMS Labs for re-analysis at CFSRE for sample mining, a process that allows for discovery of analytes not targeted within the initial scope of testing. Extracts were analyzed using a Sciex TripleTOF® 5600+ quadrupole time-of-flight mass spectrometer coupled with a Shimadzu Nexera XR ultra high performance liquid chromatograph. SWATH™ acquisition was used for isolation of product ions following the acquisition of precursor ions by TOF MS scan. This non-targeted analytical approach allows for complex drug characterization and novel psychoactive substance (NPS) discovery.

Results: 4F-MDMB-BINACA (Figure 1) was first identified in a forensic toxicology blood extract through sample mining in January 2019, following receipt of standard reference material and incorporation into the library database. Subsequently, data mining of previously acquired data revealed that the first analytical detection was in November 2018, around the same time as first identification in national seized drug reports. To date, 4F-MDMB-BINACA ingestion has been identified in 54 cases where blood or urine extracts were received for LC-QTOF testing (roughly 5,000 extracts have been received since early 2018). Cases were submitted from 13 states (TX [n=14], IN [n=14], PA [n=10], UT [n=3], FL [n=3], and AR, CT, NY, MI, KS, LA, and CO [n=1]) and the District of Columbia (n=3). The majority of cases were MDI (n=37), but cases were also submitted from DUID investigations (n=12) and with unknown circumstances (n=5). Forty-four of the individuals were male and 4 were female; sex in 6 cases was unknown. 4F-MDMB-BINACA was found in combination with 5F-MDMB-PICA (35%) and APP-BINACA (15%), another emergent synthetic cannabinoid.

The in vivo metabolism of 4F-MDMB-BINACA was investigated based on the previously reported metabolism of 5F-ADB and 5F-MDMB-PICA using a data mining approach. This resulted in the identification of 9 metabolites, discovered using four representative urine samples and four representative blood samples. The two major metabolites identified were 4F-MDMB-BINACA 3,3-dimethylbutanoic acid and 4-OH-MDMB-BINACA (Figure 1). Further data mining revealed the presence of 4F-MDMB-BINACA 3,3-dimethylbutanoic acid in 9 total cases from blood (n=6) and urine (n=3) samples. Urine results were found in the absence of parent compound.

Conclusion/Discussion: Forensic toxicologists should be aware of the emergent synthetic cannabinoid 4F-MDMB-BINACA, as its popularity continues to increase. In May and June of 2019, 4F-MDMB-BINACA was the most prevalent synthetic cannabinoid detected based on our findings. Laboratories should consider addition of 4F-MDMB-BINACA to blood testing procedures and 4F-MDMB-BINACA 3,3-dimethylbutanoic acid to urine testing procedures. Sample mining and data mining procedures for the discovery of drugs and/or NPS missed during standard targeted analysis should be more widely implemented.
Background/Introduction: Designer benzodiazepines (DBZD) are rapidly increasing as drugs of abuse; typically repurposed from pharmaceutical development, they have appeared in online shops as “research chemicals” or clandestinely sold in counterfeit pharmaceuticals. Such substances are potentially more harmful than pharmaceutical benzodiazepines, having unknown pharmacological/toxicological profiles and have added to the complexity and dangers of the illicit market for benzodiazepines. Phenazepam and etizolam were some of the first DBZD to appear in the illicit drug market in the US. Other compounds that have been reported from this class include flubromazepam, flubromazolam, delorazepam, diclazepam and clonazolam.

Designer benzodiazepines are agonists at the GABA<sub>A</sub> receptor and potentiate the inhibitory action of gamma-amino butyric acid (GABA). Benzodiazepines as a class of CNS depressants have anxiolytic, sedative-hypnotic, muscle relaxant, and anticonvulsant properties, and are often prescribed as anxiolytics, anesthetic adjuncts, and treatment for obsessive-compulsive disorders. The phenyl group appears to be a requirement for benzodiazepine activity; structural modifications of the benzodiazepine structure affects both the potency and duration of action.

Objectives: This presentation describes the positivity of DBZD, a class of novel psychoactive substances (NPS), in forensic toxicology casework between 2012 and 2018.

Methods: Testing for DBZD has evolved over time in response to the emergence of a substance, in addition to improvements in testing capabilities. Screening for DBZD typically occurs using Liquid Chromatography/Time of Flight Mass Spectrometry (LC-TOF/MS). Confirmatory analysis for phenazepam and etizolam were conducted by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) but initial results were reported qualitatively. Subsequently, a quantitative LC-MS/MS panel for 11 DBZD was developed and validated. Reporting limits range from 2 ng/mL (etizolam, flubromazolam), 5 ng/mL (clonazolam, diclazepam, delorazepam, meclonazepam, and bromazepam) to 20 ng/mL (flubromazepam and phenazepam). Between 2012 and Q1 2019, data from routine toxicological testing, including death investigation and driving under the influence (DUI) casework, was compiled to investigate positivity rates of DBZD in blood specimens.

Results: Between 2012 and 2017, there were 58 qualitative phenazepam confirmations in blood. Etizolam was added to the scope of testing in 2015; 94 qualitative results were reported between 2015 and 2017.

A directed quantitative assay for 11 DBZD was launched in October 2016, with all blood confirmations migrated to the quantitative test by January 2018. Between 2016 and Q1 2019, a total of 742 positive DBZD results were reported in 609 blood samples under the quantitative procedure. Etizolam accounted for 49% of the positive findings (98.8% were reported at or above the reporting limit of 2 ng/mL). Other positive findings were as follows: flubromazolam (n=110), delorazepam (n=103), diclazepam (n=57), bromazepam (n=40), flubromazepam (n=34), clonazolam (n=29), phenazepam (n=4) and meclonazepam (n=2). 14.4% cases reported more than 1 DBZD; 10 cases reported more than 4 DBZD, with one case reporting 6 different substances. A common finding is a combination of delorazepam and diclazepam (n=34), since diclazepam metabolizes to delorazepam, which then metabolizes further to lorazepam, which is also available by prescription.

In 2018 alone, DBZD were confirmed in 45 blood samples obtained from driving under the influence casework; 23 of those cases involved etizolam and 13 included flubromazolam.

Conclusion/Discussion: DBZD are a group of NPS that are routinely abused; their appeal lies in possessing the same effects as benzodiazepines such as alprazolam or diazepam, but typically circumventing routine drug testing. Many laboratories have limited testing capabilities for DBZD, but proper investigation may help assist in identifying cases in which DBZD may be present. DBZD have cross-reactivity with commercial benzodiazepine immunoassays; unconfirmed immunoassay screens should be evaluated for the presence of DBZD. It is important for forensic laboratories to include designer benzodiazepines within their scope of testing, especially for laboratories that perform testing for DUI cases.
Background/Introduction: Clonazolam is a novel designer benzodiazepine that bears structural resemblance to clonazepam, but modified with a triazolo ring moiety. In recent months, the northern Virginia (NoVA) area has seen a number of cases involving this drug. As there is minimal literature available on the toxicology of clonazolam, it is currently difficult to assess the impact of toxicological findings involving this drug.

Objectives: This presentation describes an investigation initiated to better understand and characterize the toxicological impact of clonazolam on human performance and postmortem casework in the NoVA area. Presented findings will include quantitative determinations of clonazolam and its metabolite, 7-aminoclonazolam. These findings will also be correlated to corresponding observations and additional toxicological findings in casework.

Methods: Analysis of clonazolam and 7-aminoclonazolam was achieved using solid phase extraction. Briefly, samples were buffered using 0.1 M phosphate buffer (pH 6) and added to prepared SPE cartridges (UCT CleanScreen ZSDAU020). SPE columns were prepared through washes of methanol and 0.1 M phosphate buffer (pH 6) prior to sample addition. After sample addition, columns were washed with water and 0.1 M acetate buffer (pH 4), then eluted with methanol followed by column drying and further eluted with 78:20:2 v/v/v dichloromethane:isopropanol:ammonium hydroxide. Combined eluents were acidified using 0.2% HCl in isopropanol and evaporated to dryness under nitrogen. Samples were reconstituted in mobile phase (98:2 v/v water:methanol with 0.1% formic acid) and analyzed via LCMSMS using multiple reaction monitoring (MRM). The calibration range for clonazolam and 7-aminoclonazolam was 0.00025 – 0.015 mg/L. Numerical values obtained using this method were considered semiquantitative as the method was not fully validated according to SWGTOX guidelines for quantitative determinations.

Results: Specimens of whole blood submitted for toxicological testing were determined to have clonazolam concentrations ranging from 0.0019 – 0.013 mg/L. Each case positive for clonazolam was also identified as containing 7-aminoclonazolam. Numerous cases among those analyzed were determined to involve poly-drug use. The most commonly co-detected drugs included THC and its metabolite (THC-COOH; 71%) and alprazolam (29%). Demographics associated with clonazolam use are displayed in the table below:

<table>
<thead>
<tr>
<th>Age</th>
<th>% of cases</th>
<th>Race</th>
<th>% of cases</th>
<th>Gender</th>
<th>% of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-20</td>
<td>64</td>
<td>White</td>
<td>57</td>
<td>Male</td>
<td>57</td>
</tr>
<tr>
<td>21-25</td>
<td>14</td>
<td>Black</td>
<td>21</td>
<td>Female</td>
<td>36</td>
</tr>
<tr>
<td>26-30</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30+</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The final presentation will contain updated information as additional data are collected.

Conclusion/Discussion: The data collected during this investigation has given insight into the toxicological impact of the potent designer benzodiazepine clonazolam. Results indicate that clonazolam concentrations in the µg/L range are potentially toxicologically relevant. This suggests a potency approximately an order of magnitude higher than other benzodiazepines commonly encountered in casework (e.g., alprazolam, clonazepam, lorazepam). Side effects associated with clonazolam use appear to be consistent with typical benzodiazepine toxicopharmacology: sedation, lethargy, incoordination, slowed reactions, and slurred speech. While polypharmacy was present in many of the cases investigated, the overall circumstances of the cases demonstrate side effects that are more severe than the other toxicological findings would generally be expected to elicit, demonstrating the impact of clonazolam. Another interesting facet of this research were the statements made to law enforcement of having consumed other benzodiazepines (i.e., alprazolam, clonazepam). While it is difficult to parse out the veracity of statements made by those under investigation for illegal activity, it does suggest the potential for unknowing consumption of a more potent benzodiazepine than the user intended.
S29: A Portrait of GHB Consumption, One of the Four Most Prevalent DUID Findings in Québec (Canada)

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2Department of Chemistry & Biochemistry, Concordia University, Montréal, Québec, Canada

Background/Introduction: In 2008, a modification to the Criminal Code introduced the International Drug Evaluation and Classification Program (DECP) in Canada to address the problem of driving under the influence of drugs (DUID). Under this program, suspected impaired drivers are arrested and undergo a 12-step evaluation that ends with a biological sample collection. Toxicological analysis of these samples throughout the years revealed a widespread prevalence of gamma-hydroxybutyrate (GHB) in cases from the province of Québec. GHB is a depressant of the central nervous system (CNS) known to impair driving ability. It can be prescribed to treat narcolepsy and other sleep disorders (Xyrem®) or taken as a drug of abuse (juice, liquid E).

Objectives: DECP cases analyzed in 2018 in the province of Québec (Canada) were reviewed to determine the demographics of GHB abuse and driving.

Methods: Biological samples (98.7% urine) were stored at 4°C pending completion of toxicological analyses. Following extraction by protein precipitation, samples were submitted to a targeted screening by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS 5500 QTRAP®, Sciex). 144 compounds were analyzed quantitatively or qualitatively in this method, including GHB. Confirmation and quantification of GHB was carried out by gas chromatography coupled to mass spectrometry (GC-MS) on samples derivatized with BSTFA+TMCS (99:1, Cerilliant). If relevant, general unknown screening was carried out by solid phase extraction (Oasis® HLB, Waters) followed by gas chromatography coupled with mass spectrometry and a nitrogen-phosphorous detector, which has a selective sensitivity for nitrogen and phosphorus containing compounds (GC-MS/NPD, 7890/5975 MSD, Agilent). All quantitative methods were validated under ISO 17025:2005, CAN-P-1578 and SWGTOX guidelines. To ensure positive reports of only exogenous GHB consumption cases, the positivity threshold was set at 25 mg/L in urine and 10 mg/L in blood. Data were compiled for all 2018 DECP cases, including gender and age of the suspect, time and location of the event, and detected drugs.

Results: In 2018, GHB was the fourth most common finding in Québec’s DECP cases, following methamphetamine, cannabis and cocaine. Out of the 790 samples collected and analyzed that year, 21% (n = 162) tested positive for GHB. Prevalence varied within the 17 administrative regions of the province, ranging from 0% to 38%. Younger males (16-34 years old) accounted for 30% of all GHB cases, an overrepresentation with regards to the general population (14%) similar to the one found in all DUID arrests (41%). Toxicological analyses revealed that co-consumption of GHB with other psychoactive substances was the norm, with only 6 cases (3.7%) where no other psychoactive substance was found. The level of co-consumption for this substance is greater than for overall DECP cases (76%). In that single year, 24 cases (15%) were the result of repeat offenders, with one individual being caught 4 times just in the first half of the year.

Conclusion/Discussion: Abuse of the CNS depressant GHB causes euphoria, relaxation, drowsiness and sedation, which impairs driving ability. Data presented here show a surprisingly elevated GHB prevalence amongst impaired drivers of the province of Québec (Canada). These data correlate well with seized drugs analysis by Health Canada’s Drug Analysis Service, which shows that GHB is one of the top five controlled substances identified in Québec but no other province. To our knowledge, no other jurisdiction worldwide has reported such a widespread presence of GHB in forensic toxicology casework. However, several laboratories do not routinely screen for GHB in DUID cases, assuming negligible prevalence. This work highlights the imperativeness of including GHB in routine screening in selected jurisdictions where the consumption habits warrants it.
Background/Introduction: Buprenorphine is a semisynthetic mixed agonist/antagonist opioid that is 25 - 40 times stronger than morphine, effective by non-parenteral administration and has a long half-life. These properties make it an effective drug to treat opioid dependence. The current opioid epidemic has made buprenorphine drug dependence therapy common and its detection in driving-under-the-influence of drugs (DUID) cases frequent. Buprenorphine was recently promoted to a Tier I drug in the 2017 Update of the Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicles Fatalities.

Objective: To investigate buprenorphine and norbuprenorphine blood concentrations, other drugs detected with buprenorphine, compare blood concentrations with driving performance, behavior, and field sobriety tests in DUID cases in Southwestern Virginia.

Methods: The Virginia Department of Forensic Science Western Toxicology Laboratory began confirmation and quantification of buprenorphine in blood for DUID cases in August 2017. Cases that screened presumptive positive for buprenorphine by ELISA using Immunalysis reagents were extracted with UCT ZSDAU020 SPE columns and quantified using an Agilent 1290 liquid chromatograph and 6460 tandem mass spectrometer. The method was validated according to SWGTOX guidelines. Case histories were collected from evidence submission documents that contained officer notes or interactions between officer, attorney and toxicologist at court.

Results: The linear range for buprenorphine and norbuprenorphine is 0.5 to 20 ug/L. 111 cases of buprenorphine and 110 cases norbuprenorphine quantified within the linear range. Blood buprenorphine concentrations ranged from 0.54 to 9.3 ug/L and norbuprenorphine concentrations ranged from 0.55 to greater than 20 ug/L. The median buprenorphine blood concentration was 1.9 ug/L and norbuprenorphine 2.5 ug/L. The median buprenorphine to norbuprenorphine ratio was 0.7 (range 0.1 – 5.8). Buprenorphine was frequently found with other drugs. Only 14 of 111 cases did not contain additional drugs or alcohol that were included in testing panels. The most common drug classes found with buprenorphine were benzodiazepines, amphetamines and cannabinoids.

<table>
<thead>
<tr>
<th></th>
<th>Benzo</th>
<th>Opioid</th>
<th>Meth/Amp</th>
<th>Coc/BE</th>
<th>THC/THCA</th>
<th>Ethanol</th>
<th>Others</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>61</td>
<td>5</td>
<td>41</td>
<td>6</td>
<td>22</td>
<td>4</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>%</td>
<td>55.0</td>
<td>4.5</td>
<td>36.9</td>
<td>5.4</td>
<td>19.8</td>
<td>3.6</td>
<td>18.0</td>
<td>12.6</td>
</tr>
</tbody>
</table>

In cases with available history, buprenorphine was frequently prescribed; however, abused buprenorphine by sublingual, insufflation and parenteral routes of administration was also found. Selected case histories will be presented.

Conclusion/Discussion: The detection of buprenorphine in DUID cases requires a sensitive analytical procedure capable of measuring blood concentrations in the 0.50 to 1.0 ug/L range. Approximately one-third of the cases had buprenorphine blood concentrations less than 1.0 ug/L. Reported driving observations include weaving within lanes, crossing center-lines, failure to follow traffic signs and loss of vehicle control. Typical central nervous system depressant effects such as drowsiness, lethargic behavior, slow reaction time and poor coordination were commonly observed along with pinpoint pupils. No correlation between buprenorphine blood concentration and observed symptoms or performance on DRE or field sobriety tests could be determined. The high number of cases with additional drugs detected add to the complexity of evaluating buprenorphine driving impairment. As with most DUID cases, drugged driving impairment with buprenorphine is determined by poor driving performance, driver behavioral observations, performance on DRE and field sobriety tests and supportive toxicology findings.

Keywords: Buprenorphine, DUID, Human Performance
S31: Curious Cases of Cannabis Use in Colorado

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Background/Introduction: At the Colorado Bureau of Investigation, approximately 70% of DUI cases that request drug toxicology screen positive for cannabinoids and approximately 60% confirm positive for delta-9-tetrahydrocannabinol (THC). Colorado voters first legalized medical marijuana in November 2000 and recreational marijuana in November 2012. A permissible inference law of 5 ng/mL THC was signed into law in May 2013 which allowed a jury instruction that a subject at or above this concentration may be considered to be under the influence of one or more drugs.

Due to the nature of Colorado's laws and the readily availability of cannabis products, the potential for chronic users to operate a motor vehicle has increased. As a result, the concentrations of THC, when evaluated in context with case information, may lead to surprising interpretations and jury verdicts.

Objectives: To highlight three cases involving operating a motor vehicle in the State of Colorado with an unusual combination of driving behavior, Standardized Field Sobriety Test (SFST) performance, blood THC results, and jury verdicts.

Methods: Cases were initially screened by ELISA and confirmed utilizing a liquid/liquid extraction and analyzed by LC/MS/MS for the Cannabinoid analytes THC, THC-OH, and THC-COOH. A set of six calibrators from 1.0-50 ng/ml (THC, THC-OH) and 5.0-250 ng/ml (THC-COOH) and 3 controls were concurrently analyzed with all casework. The measurement uncertainty is 19.2%.

Results: Due to the Colorado Expressed Consent Law, each of the three cases had blood samples collected within one hour of the incident time. Case 1 involved a medical marijuana user that last admitted cannabis consumption 1.5 hours prior to the stop for speeding. Case 2 involved a chronic marijuana user for PTSD that last admitted cannabis consumption 5.5 hours prior to the stop for not having license plates. Case 3 involved a driver who was stopped at an intersection and was rear-ended by a driver that suffered serious injuries. The subject in Case 3 claimed no cannabis use or drug use. A summary of the toxicology results and SFST findings are as follows:

<table>
<thead>
<tr>
<th>Toxicology Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng/mL THC</td>
</tr>
<tr>
<td>Case 1</td>
</tr>
<tr>
<td>Case 2</td>
</tr>
<tr>
<td>Case 3</td>
</tr>
</tbody>
</table>

SFST Findings

<table>
<thead>
<tr>
<th>SFST Findings</th>
<th>Full DRE Evaluation</th>
<th>HGN</th>
<th>WAT</th>
<th>OLS</th>
<th>LOC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>No</td>
<td>0/6</td>
<td>2/8</td>
<td>1/4</td>
<td>Present - Left</td>
</tr>
<tr>
<td>Case 2</td>
<td>No</td>
<td>6/6</td>
<td>1/8</td>
<td>1/4</td>
<td>Not Performed</td>
</tr>
<tr>
<td>Case 3</td>
<td>Yes</td>
<td>0/0</td>
<td>0/8</td>
<td>0/4</td>
<td>Absent</td>
</tr>
</tbody>
</table>

The subjects in Cases 1 and 2 were acquitted at trial. There is no legal update for Case 3.

Conclusion/Discussion: These three case studies exemplify the evolving landscape of cannabis use in the state of Colorado. When the toxicology results are evaluated in context with the observed driving behavior and SFST performance, the indications for impairment are not always clear cut. It can be a challenge to assess the impact of marijuana legalization on impaired driving in light of chronic cannabis use.
S32: Buprenorphine in Wisconsin Drivers

Lorraine D. Edwards*

University of Wisconsin State Laboratory of Hygiene, School of Medicine and Public Health, Madison, WI

Background/Introduction: As the opioid epidemic continues in Wisconsin and throughout the United States, the use of buprenorphine (BUP) as a form of medically assisted treatment continues to increase. As a result of the increase in detection by laboratories, BUP has been moved from Tier II to Tier I on the recommendation of the National Safety Council’s Alcohol, Drugs and Impairment Division. Relatively little information on the observed effects of BUP, outside a laboratory or a controlled driving course, exist in the literature. Opinions of the medical community, however, vary on whether or not BUP can be abused, diverted and cause impairment in drivers. The Wisconsin State Laboratory of Hygiene (WSLH) has seen an increase in the number of driving cases where BUP is listed as a suspected drug and subsequently detected in the blood of impaired drivers. In 2017, norbuprenorphine (NBUP), the pharmacologically active metabolite of BUP, was one of the top 20 most frequently detected drugs in Wisconsin’s Operating While Intoxicated (OWI) casework.

Objectives: The WSLH monitored the detection of BUP and NBUP in Wisconsin drivers over a 2 year period. The objectives of this data evaluation were to 1) determine the number of Wisconsin drivers operating under the influence of BUP and/or NBUP; 2) characterize indicators of impairment observed when drivers were under the influence of BUP and/or NBUP; 3) document the frequency of drivers operating under the influence drugs in addition to BUP and/or NBUP.

Methods: Forensic toxicology data from 2012 to 2017 was compiled for Wisconsin drivers operating under the influence of drugs. Data from approximately 19,000 drivers each year was reviewed for cases where BUP and/or NBUP were reported. Drugs reported in addition to BUP and/or NBUP were documented. To characterize the impairing effects of BUP and/or NBUP, police reports and Drug Recognition Expert (DRE) evaluations were obtained for any cases where BUP and/or NBUP were the only drug(s) reported.

Results: A total of 204 individuals (78 females, 126 males) were driving under the influence of BUP and/or NBUP between 2012 and 2017. Three cases from 2018 were included in the data set. Concentrations of BUP and NBUP in whole blood (ng/mL) ranged (mean) from 0.6 to 14 (2.0) and 0.5 to 20 (2.1), respectively. Benzodiazepines, a category of drugs considered to elevate the risk of central nervous system depression when coadministered with BUP, was detected in 70% of the drivers, followed by amphetamine like stimulants (21%), other opioids (20%), ethanol (8%) and cocaine (7%). Only four of the drivers (2%) included in this data set were operating under the influence of either BUP and/or NBUP alone. Physical and behavioral observations made by law enforcement were inconsistent with those typically reported for drivers under the influence of an opioid. Indicators of impairment varied and included a combination of narcotic analgesic, central nervous system (CNS) depressant and stimulant like effects.

Conclusion/Discussion: Poly drug use is extremely prevalent in Wisconsin drivers, so prevalent that only four of the 204 cases evaluated had BUP and/or NBUP as the only drug(s) detected. Law enforcement made contact with three of the four subjects due to either a crash or poor/reckless driving. Poly drug use in drivers makes it difficult to evaluate the impairing effects of a single drug. While the number of cases reported here is limited, the results demonstrate the complex paradigm associated with forensic interpretation of BUP in OWI casework and the frequency of poly drug administration in Wisconsin drivers. Several cases where BUP and/or NBUP were reported in combination with benzodiazepines and amphetamine like stimulants will be discussed.
S33: Everything is Bigger in Texas: A Look at Alcohol-Impaired Driving Trends in the City of Houston (2014 – 2018)

Melissa Lloyd, Corissa Rodgers*, Peter Stout, Dayong Lee
Houston Forensic Science Center, Inc., Houston, Texas

Background/Introduction: “Everything’s bigger in Texas.” Unfortunately, this adage holds true for traffic fatalities in the state. Houston is in Texas’ largest county, Harris, which in 2017 saw 456 of the state’s 3,722 traffic fatalities as reported by the National Highway Traffic Safety Administration (NHTSA). Of those, 44% were classified as alcohol impaired driving fatalities. While Houston’s population from 2014 to 2018 remained steady at 2.2 to 2.3 million residents, requests for alcohol analysis submitted to Houston Forensic Science Center (HFSC) for impaired driving offenses doubled.

Objectives: This study examined alcohol analysis results and case information associated with impaired driving arrests in Houston between 2014 and 2018. Numbers of cases, distribution of blood alcohol concentrations, and demographics were examined. These analyses were intended to provide a better understanding of the impaired driver population in Houston and identify trends observed over the past five years.

Methods: The fatal and non-fatal impaired driving cases analyzed by HFSC for ethanol in blood samples with offense dates falling between January 1, 2014 and December 31, 2018 were included. Blood samples were collected by Houston Police Department from drivers as indicated in the laboratory information management system. Cases were analyzed for ethanol by headspace gas chromatography interfaced with dual flame ionization detection. The limit of quantification (LOQ) was 0.010 g/dL and range of linearity was 0.010-0.500 g/dl for ethanol (LOQ was 0.02 g/dL in 2014). Blood alcohol concentrations (BAC) and demographic data including age (<21, 21-44, 45-65, and >65 years), sex, and race/ethnicity were evaluated. Drug screen and confirmatory analyses were performed for cases with BAC <0.10 g/dL and fatality cases, using enzyme-linked immunosorbent assay and gas/liquid chromatography-mass spectrometry; approximately 50% of drug confirmation analyses were performed by external laboratories. Data were analyzed using Microsoft Excel 2016.

Results: Over the five-year period examined, 11,372 blood samples were analyzed by HFSC as part of impaired driving investigations, with a mean (median) BAC of 0.167 g/dL (0.176 g/dL) and age of 36.4 (34) years. Of the suspected impaired drivers, 80% were male and 19% were female, with no significant difference in mean BAC between the two groups (ANOVA; p=0.45). The mean BACs for drivers younger than 21, 21-44, 45-65 and older than 65 were 0.116 g/dL (25% of cases were negative for ethanol), 0.165 g/dL, 0.177 g/dL, and 0.160 g/dL, respectively. Most drivers were White (64%) with a mean BAC of 0.173 g/dL; 20% of drivers were Black (mean 0.144 g/dL), 5% Hispanic (0.176 g/dL), and 3% Asian (0.169 g/dL). Overall mean BAC decreased between 2014 and 2018, from 0.181 g/dL to 0.157 g/dL, and this trend was attributed to the increasing number of cases received wherein no ethanol was detected (ethanol <LOQ), from 15 cases in 2014 to 445 cases in 2018. Drug prevalence in the ethanol-negative cases was examined; the six most prevalent drugs detected in ethanol-negative cases from 2014 to mid-2018 (some confirmation testing still pending) were cannabinoids (39%), phencyclidine (29%), alprazolam/metabolite (26%), cocaine/metabolites (14%), carisoprodol/meprobamate (14%), and hydrocodone (11%).

Conclusion/Discussion: NHTSA reports from 2014 to 2017 showed Texas having the nation’s highest number of alcohol-impaired driving fatalities. In Houston, drugged driving is on the rise as well, evidenced by the increased number of cases received that were negative for alcohol but found to contain one or more drugs. Polysubstance cases, here defined as samples confirmed to contain two or more classes of drugs, rose from <0.2% of cases negative for ethanol in 2014 to >5% in 2017. Such data can aid in raising public awareness and designing preventative measures to reduce impaired driving in all its forms in the city of Houston.
S34: Alabama’s DUID Oral Fluid Drug Testing Program – One Year Review


Alabama Department of Forensic Sciences.

Background/Introduction: Oral fluid (OF) drug testing in DUID cases has many advantages including rapid, non-invasive collection, ability to collect the specimen proximate to time of driving, and the presence of active, parent drug that likely reflects recent drug use. In August of 2018, the Alabama Department of Forensic Science (ADFS) approved three roadside screening devices (i.e. Draeger DT5000, Alere DDS2, Randox Evidence MultiSTAT) and validated in-house oral fluid evidentiary, confirmation methods.

Objectives: Highlight the results from OF DUID cases analyzed from inception thru 2019 and discuss challenges, lessons, and improvement made to the program over the first year of implementation.

Methods: Roadside OF screening conducted by law enforcement to establish probable cause was conducted using Draeger DT5000 or Alere DDS2 devices. Quantisal® OF collection devices with volume adequacy indicators were used for evidentiary collection. OF Confirmation (evidentiary) testing was conducted using two validated methods on an Agilent 6460 LC/MS/MS. The first method identified 20 drugs of abuse following an in-tip Dispersive Pipette extraction (DPX) technique. In the second method, the extraction of six cannabinoids: THC, THC-OH, THC-COOH, CBN, CBD, and CBG were performed by liquid-liquid extraction. OF scope and cutoffs were selected based on NSC-ADID recommendations for toxicological investigations of drug-impaired driving cases. Accompanying blood specimens were screened by Tecan Evo 75 using Immunalysis reagents or a Randox Evidence Analyzer. Blood confirmations were performed by liquid-liquid or solid phase extraction and GC/MS or LC/MS/MS. Positivity rate, median concentration, and prevalence for three of the most commonly detected drugs (i.e. cannabinoids, alprazolam, and methamphetamine) in OF and blood were determined.

Results: Roadside OF screening devices have been purchased by six agencies. During the first year of the program, select law enforcement agencies and DREs were equipped with Quantisal® collection devices. Approximately, 20 OF DUID submissions have been received through May 2019 with 85% of those cases containing both oral fluid and blood specimens. THC was detected at a higher positivity rate (10/10 = 100%, LOD = 0.5 ng/mL) in OF specimens than blood specimens (3/7 = 43%, LOD = 0.5 ng/mL). Median THC concentrations were 3X higher in OF. Methamphetamine had the same positivity rate (8/9 = 89%) between OF (LOD = 20 ng/mL) and blood (LOD = 5 ng/mL). Alprazolam, despite its known low partitioning into the OF, had the same positivity rate in OF (LOD = 1 ng/mL) and blood (LOD = 1 ng/mL). However, OF alprazolam concentrations were 7X lower than blood concentrations.

Conclusion/Discussion: Alabama is the first crime laboratory to implement a statewide OF program by offering approved roadside OF screening devices and confirmation OF testing. The high parent drug positivity rate in OF makes this specimen an attractive option for DUID testing. This is likely due in part to OF collection close to the time of arrest or crash when compared to blood. However, the combined analysis of blood and OF paints the most comprehensive picture of drug use and a potential explanation and cause for impairment at the roadside. We have recently redesigned our DUI biological specimen kits for statewide distribution. These kits will be available in late 2019. At which point, officers will collect two tubes of blood and one oral fluid specimen for all DUI cases.
Background/Introduction: As cannabis is increasingly being legalized for both medicinal and recreational purposes, there is a critical need to find a marker for cannabis impairment. The pharmacokinetics and associated pharmacodynamics of cannabis administered via vaporization and oral consumption are currently not well understood and need to be better defined to evaluate methods of determining whether or not an individual under the influence of cannabis is impaired. The purpose of this study was to develop valid and reliable measures of impairment among individuals acutely exposed to orally ingested and vaporized cannabis. Data presented will include outcomes from a battery of cognitive and psychomotor performance assessments, field sobriety tests, subjective drug effect ratings, and analyses of cannabinoid and non-cannabinoid candidate biomarkers measured in blood, oral fluid and urine specimens obtained in parallel with pharmacodynamic measures.

Objectives:

1.) Understand the dose effects of acute oral and vaporized cannabis administration on measures of cognitive/psychomotor performance assessments and field sobriety tests.

2.) Understand which biomarkers in oral fluid and blood and which field sobriety tests are, and are not, predictive of impairment on measures of cognitive performance and functioning following cannabis exposure.

3.) Understand the comparative pharmacokinetic and pharmacodynamic profiles of oral versus vaporized cannabis.

Methods: Twenty individuals who had not used cannabis for at least 30 days participated in six, double-blind, experimental sessions that were separated by at least 1 week. Across all six sessions each participant consumed cannabis brownies containing 0, 10, or 25mg THC or inhaled vaporized cannabis containing 0, 5, or 20mg THC. During each session, blood, oral fluid, and urine were collected and subjective, cognitive, and psychomotor effects were assessed before cannabis administration and for 8hrs thereafter. A variety of field sobriety tests were also administered. Blood, oral fluid, and urine were tested for THC and its primary metabolites using targeted LC-MS/MS. Blood samples also underwent an exploratory screen for possible new biomarkers of impairment.

Results: Subjective drug effects were generally dose-orderly within each route of administration with peak effects being lower and delayed after oral ingestion compared to vaporized cannabis inhalation. Oral dosing of 10 and 25mg, and 20mg vaporized THC doses impaired cognitive/psychomotor performance, but 5mg vaporized cannabis produced discriminative drug effects without impairment. Pharmacokinetic measures indicate target compound profiles were also dose-orderly and route dependent. For blood and oral fluid: 1) THC-COOH concentrations were higher after oral consumption compared to vaporized; 2) THC was higher after vaporization. Blood 11-OH-THC concentrations were higher after oral consumption. THC was highest in oral fluid for both administration routes, and the highest levels in blood for vaporization. Neither THC, THC-COOH, 11-OH-THC, CBD, nor CBN were reliable markers of impairment in blood or oral fluid for either route of administration. Field sobriety tests including walk and turn, horizontal gaze nystagmus, one leg stand, and Romberg balance did not reliably discriminate between impaired and unimpaired individuals.

Conclusion/Discussion: The current understanding of pharmacokinetic and pharmacodynamic characteristics of cannabis administered via vaporization and oral consumption is limited. A greater understanding of these parameters will help determine whether or not an individual under the influence of cannabis is impaired. Our work indicates that THC is not a reliable marker of cannabis impairment.
S36: Quantification of Cannabinoids in Oral Fluid, Breath, and Whole Blood to Evaluate Markers of Recent Cannabis Smoking and Driving Impairment

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Background/Introduction: With legalization of recreational and medical cannabis occurring in 10 and 33 states, respectively, cannabis usage is on the rise. Increased prevalence of cannabis consumption and subsequent intoxication resulting in impaired driving has led to a growing public safety concern. Recent population-based studies examining motor vehicle crash reports regarding cannabis have been inconclusive or shown moderately increased risk of crashing. With legalization comes the responsibility of developing methods to distinguish which markers correlate with recent cannabis use, but not past use, and to determine cutoff levels that correlate with driving impairment. As a result, many states have adopted per se driving laws, which range from zero to specific concentration limits (e.g. < 5 ng/mL THC) in whole blood. These laws have numerous caveats, including detection of THC above per se cutoffs in blood of chronic cannabis users when not impaired and the challenges of roadside blood collection. THC concentrations can drop up to 90% in the time it takes from when an impaired driver is pulled over to when their blood is drawn. This underscores the need to pursue matrices with less invasive collection, such as oral fluid and exhaled breath.

Objectives: The overall goal of this study, which is funded by California assembly bill 266, is to evaluate the impact of smoking cannabis on driving performance. As part of the larger project, we aim to determine which cannabinoids or metabolites are the strongest markers of recent cannabis use in whole blood, oral fluid, and breath.

Methods: Liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays to measure ∆9-tetrahydrocannabinol (THC), 11-hydroxy-∆9-THC (11-OH-THC), 11-nor-9-carboxy-∆9-THC (THC-COOH), 11-nor-9-carboxy-∆9-THC glucuronide (THC-COOH-gluc), ∆9-THC glucuronide (THC-gluc), cannabiol (CBN), cannabidiol (CBD), cannabigerol (CBG), tetrahydrocannabiverin (THCV), and ∆9-tetrahydrocannabinolic acid A (THCA) in whole blood, oral fluid, and breath (THC only) were developed and validated. Oral fluid was collected using the Quantisal device and sample preparation of both oral fluid and whole blood used solid phase extraction (Oasis Prime HLB plates, Waters). Breath samples were collected using the SensAbues device and extracted directly from the device with methanol. Cannabinoids were separated on a BEH C18 column and detected by a TQ-S micro tandem MS (Waters), with a total run time of 5 min per sample.

Study participants (n = 184), either frequent or infrequent users, smoked a cannabis joint with THC concentrations of ~0, 6%, or 13% and were monitored for ~6 hours following smoking. For each participant, the following samples were collected over the specified time range: 5 oral fluid, 5 breath, and 9 whole blood. LC-MS/MS measurements were applied to these samples.

Results: Lower limits of quantification for all analytes were either 0.4 or 1 ng/mL in oral fluid, 0.5 to 2 ng/mL in whole blood, and 80 pg/pad for THC in breath. Inter- and intra-day precision assessment demonstrated CVs from 2 – 15% for all analytes in oral fluid. Matrix interferences for all analytes resulted in biases not exceeding ±20%. Patterns of cannabinoids and metabolite concentrations following smoking were evaluated. Sensitivity, specificity, and positive and negative predictive values for each analyte in all three matrices were assessed.

Conclusion/Discussion: Results demonstrate that THC in oral fluid is a promising marker for detection of recent cannabis use. The breath collection device has limitations resulting in reduced sensitivity; consistent detection of THC is not possible beyond 1 hour after smoking. This study is an important step in scientifically determining if specific cutoff concentrations can be validated to support cannabis DUI per se laws. The long-term goals of this study are to improve road safety while minimizing persecution of non-impaired cannabis users.
S37: New Benzodiazepine Variants in Recent Casework

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Background/Introduction: Benzodiazepines are among the most potent GABA receptor modulators prescribed for anxiety. The most commonly prescribed benzodiazepines include alprazolam, diazepam, and clonazepam. Dosages and effects of these drugs are well-known, however, within the last year a new group of unapproved benzodiazepines have appeared with potencies and effects that remain largely unknown. While traditional benzodiazepine immunoassays often cross-react with these new benzodiazepine variants, subsequent tandem mass spectrometry confirmations may overlook these compounds if they are not part of the acquisition method.

Objectives: This presentation raises awareness about the new types of emerging benzodiazepines, and the capabilities needed to identify and confirm their presence in toxicology specimens.

Methods: Toxicological screening was performed on blood and/or urine specimens by headspace gas chromatography, immunoassay, and liquid chromatography time of flight mass spectrometry (LC-QTOF/MS). Confirmatory analyses were performed by a 10 minute liquid chromatography tandem mass spectrometry (LC-MS/MS) assay with a limit of detection set at 10 ng/mL. The routine confirmation assay provides quantitative support for 9 parent drugs along with 4 metabolites, while an extended qualitative control monitors for an additional 23 compounds.

Results: We describe selected case reports where QTOF analysis revealed previously unidentified benzodiazepine derivatives, such as flualprazolam, clonazolam, and flubromazepam. In the first case, an intoxicated driver investigation of a 20-year-old female showed no ethanol in blood, but screened presumptive positive for cannabinoids and benzodiazepines. THC and THC metabolites were confirmed in blood along with alprazolam. QTOF analysis also revealed the presence of flualprazolam, a fluorinated derivative of alprazolam which was subsequently confirmed by LC-MS/MS analysis.

In an investigation of a motor vehicle accident involving a 23-year-old-male, the suspect displayed slurred speech and dilated pupils. Much like the first case, alcohol was negative and the immunoassay was presumptive positive for cannabinoids and benzodiazepines. LC-MS/MS confirmed THC-COOH in blood, but the confirmation panel of known benzodiazepines was negative. A QTOF screen showed the presence of flualprazolam in blood, which was qualitatively confirmed by LC-MS/MS analysis.

A third case identified involved a military personnel readiness investigation where an 18-year-old male arrived for duty and was witnessed to be intoxicated. He admitted to taking a Xanax tablet the night before. The immunoassay produced a presumptive positive benzodiazepine response, however alprazolam was not confirmed. A comprehensive QTOF screen revealed the presence of clonazolam in blood. The proposed metabolite 7-aminoclonazolam was synthesized and used as reference standard in urine to confirm clonazolam use by LC-MS/MS.

The final case describes a 21-year-old male who failed to report for morning duty. When contacted by telephone, he was reported to be incoherent with slurred speech. He stated that he had not been recently drinking alcohol. Blood alcohol was confirmed negative and the immunoassay was positive for benzodiazepines, however the confirmation panel was negative. QTOF analysis revealed the presence of delorazepam, alpha-hydroxyetizolam, and flubromazepam, which were confirmed by LC-MS/MS analysis.

Conclusion/Discussion: The emergence of new benzodiazepine derivatives such as flualprazolam, flubromazepam, and clonazolam underscores the need for laboratories to incorporate these compounds and their metabolites into the list of analytical capabilities. In some of the cases mentioned above, the additional benzodiazepines would have been missed without the specificity provided by high resolution mass spectrometry screening and sensitivity of tandem mass spectrometry confirmations. We recommend that presumptive positive benzodiazepine immunoassays should be pursued further especially if the confirmatory analyses are negative, due to the possibility of new and unforeseen derivatives.
S38: Trends in the Use of Psychoactive Substances Affecting Fitness to Drive in Poland Based on Routine Examination of Drivers in 2010-2018

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Background/Introduction: Driving while under the influence of drugs (DUID) and/or alcohol is still a major safety problem among many countries all over the world. Substances affecting fitness to drive include psychotropic substances of natural and synthetic origin as well as medicines. Cannabis products are the most widespread psychoactive substances (Senna 2010). Stimulants, especially amphetamines and benzodiazepines are also popular (Blencowe 2012). A very large and diverse group of new psychoactive substances (NPS), so-called “legal highs” are spreading among drivers during last few years, reaching 7% drivers presumptive positive (Wille 2018), which correlates with our findings. The most common NPS in Poland in the last year were NEH, MDMB-CHMICA, 4-FMA, 4-CMC.

Objectives: Presentation of trends in use of drugs affecting fitness to drive based upon blood tests of drivers stopped for routine inspection and from road accidents.

Methods: A blood screen for the presence of cannabinoids, amphetamines, cocaine, benzodiazepines and opiates was performed with enzyme-linked immunosorbent assay (ELISA). Coupled techniques (GC-MS and LC-MS/MS) were used for confirmatory analyses.

Results: The collected statistics refer to the period 2010-2018. These included results from 5846 roadside inspections and 3787 cases in which police indicated a road accident occurrence. In almost half of the cases THC was present in the tested blood samples, a trend has been maintained since 2011. The second most popular substance among drivers isamphetamine, detected in about 23% of cases and methamphetamine (about 6% of cases). MDMA, which has had almost disappeared from the drug market for a few years, reappeared in 2014 and its popularity among drivers is growing, exceeding 4% in 2018. Benzodiazepine derivatives (about 3%), especially clonazepam, are also popular among drivers. Since 2013, there has been an increase in number of cases in which NPS have been detected. On the basis of the attached blood protocols, no conclusions can be drawn regarding the performance of the drivers.

Conclusion/Discussion: Cannabis products are still the most popular group of psychoactive substances among drivers. Only three substances: THC,amphetamine and methamphetamine are responsible for 80% positive results of roadside DUI inspections. The stimulants (amphetamines) are particularly dangerous due to the impaired assessment of the driver’s own abilities. Our results indicate that amphetamines are involved in greater risk of participating in an accident in comparison to other substances. Cannabis products are also dangerous because of its prevalence. A very large and diverse group of NPS, so-called “legal highs” are spreading among drivers, exceeding 5% in 2015.


S39: Comparison of Extraction Methods for Novel Synthetic Opioids in Blood

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Background/Introduction: Novel synthetic opioids continue to be reported in acute and fatal intoxications. Several non-fentanyl derived synthetic opioids, including U-47700, AH-7921, and MT-45, are listed as Schedule I controlled substances by the Drug Enforcement Administration. Current reported analytical methods for the detection of these compounds utilize solid-phase extraction (SPE) and liquid-liquid extraction (LLE). Examination of varying extraction methods applied to a comprehensive method to detect these analytes could provide laboratories with alternative extraction methods to suit their needs.

Objectives: This study compares QuEChERS (quick, easy, cheap, effective, rugged, and safe), filter vials, supported liquid extraction (SLE), and LLE techniques to a validated SPE method for the analysis of 7 novel synthetic opioids in whole blood: U-47700, U-49900, U-50488, AH-7921, MT-45, W-18, and W-15. The five extraction methods were compared in terms of matrix effects, extraction recovery, solvent use, time, and cost.

Methods: Matrix effects (post-extraction addition) and extraction recovery were evaluated at a low quality control concentration (0.75 ng/mL, except 2.5 ng/mL for W-18) in different sources of whole blood in duplicate. Cerex® Clin II (3 mL, 35 mg) SPE columns (Tecan, Baldwin Park, CA), Shimadzu Micro Volume QuEChERS kit™ (Shimadzu Corporation, Kyoto, Japan), Thomson eXtreme|FV® (PVDF) filter vials (Thomson Instrument Company, Oceanside, CA), and Biotage® ISOLUTE SLE+ (2 mL) SLE columns (Fisher Scientific, Hanover Park, IL) were used for sample preparation. When performing SPE, both the acidic/neutral drug and the basic drug fractions were collected for analysis. The QuEChERS and filter vial protocols required lower amounts of matrix volume during sample preparation but drug quantity (0.375 ng) remained constant throughout each experiment. Three elution solvents were evaluated for SLE and 3 organic solvents were assessed for LLE. All samples were analyzed using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method on an Agilent 1290 Infinity II LC coupled to an Agilent 6470 triple quadrupole MS [Lowry Forensic Toxicol 2019]. Solvent use and cost were calculated for an individual extracted sample. Cost was estimated for consumable materials only, exclusive of analyst time or instrument cost. Time was estimated based on the protocol for the extraction, including sample preparation and time to dry down.

Results: Overall, SPE produced the least ionization enhancement or suppression (±18.0%) when compared to the other techniques (-55.8 – 83.8%). The four alternative extraction methods resulted in higher recoveries for the W-series analytes (23.2 – 84.3%) when compared to SPE (25.3 – 33.7%). Extraction with SLE (ethyl acetate as the elution solvent) and LLE (70/30 n-hexane/ethyl acetate as the organic phase solvent) provided comparable extraction efficiencies to the SPE method. SPE required the greatest amount of organic solvent use (8.1 mL) and total time (160 min) to complete, with the cost of consumables around $4.64 per sample. The QuEChERS and filter vial protocols required minimal organic solvent use, with 0.3 mL and 0.1 mL respectively. The filter vial method was the cheapest to perform ($2.52) and had the shortest extraction time (15 minutes).

Conclusion/Discussion: While SPE might be the better analytical choice in terms of matrix effects and extraction recovery, other methods required lower amounts of organic solvent, were cheaper to perform in terms of cost of consumables, and required less time to complete. Each method analyzed was effective in capturing synthetic opioids at low concentrations and could be beneficial depending on the desires of the laboratory.

Keywords: Novel Synthetic Opioids, Solid-Phase Extraction, Liquid-Liquid Extraction, Supported Liquid Extraction, QuEChERS
Validation of a High Throughput Screening and Quantification Method for the Determination of Gabapentinoids in Blood Using a Combination of LC-TOF-MS and LC-MS-MS

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Background/Introduction: In recent years, there has been an increasing awareness of gabapentinoid misuse among individuals with a history of polysubstance use. Both gabapentin (GP) and pregabalin (PGL) are understood to potentiate the effects of opioids, with fatalities being reported with increasing frequency.

Objectives: An efficient procedure was validated to screen and quantitate GP and PGL in blood samples using a combination of liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS) and liquid chromatography tandem mass spectrometry (LC-MS-MS). The methods provided a fast and reliable high throughput screening and confirmation testing strategy for the detection of GP and PGL in blood specimens.

Methods: A protein crash procedure using 100 µL of whole blood was validated using SWGTOX guidelines. Reversed-phase chromatographic separations were performed using a gradient with 0.1% formic acid in deionized water and in acetonitrile as mobile phases. Mass spectral data were acquired in ESI+ mode and captured using MassHunter software. The LC-TOF-MS was operated in full scan mode acquiring a mass range of 100-1700 m/z, while LC-MS-MS data was acquired using dynamic multiple reaction mode (dMRM). A total of 1,091 blood specimens were screened for GP/PGL using LC-TOF-MS and all presumptive positives were then re-scheduled for confirmation using LC-MS-MS.

Results: The limits of detection for both analytes on the LC-TOF-MS and LC-MS-MS were 0.5 mg/L and 0.1 mg/L respectively, with the LC-MS-MS method linear from 0.5-50 mg/L. A total of 101 (9.3%) blood specimens screened positive using the developed LC-TOF-MS method for GP while 13 (1.2%) blood specimens screened positive for PGL. Out of all cases that screened positive for GP and PGL, 100% were confirmed positive by LC-MS-MS. Blood concentrations of GP and PGL ranged from < 0.5 – 215 mg/L, and < 0.5 – 32 mg/L respectively. Of the blood specimens that had previously screened negative by LC-TOF-MS, 10% (N=100) were randomly selected and tested by LC-MS-MS with 100% confirmed negative for GP and PGL.

Conclusion/Discussion: It has been shown that the validated methods provide a fast and reliable testing strategy for the detection of GP and PGL in blood specimens when considering the quick sample prep and instrument runtime and reliability of the results. The validated methods provide laboratories with an alternative testing option for screening and confirmation of GP and PGL by LC-TOF-MS combined with LC-MS-MS.
Background/Introduction: Many different panels of drugs have been screened for by liquid-chromatography high-resolution mass spectrometers (LC-HR-MS/MS) including some whole blood large panel screens applied to a forensic setting. With the advantages of retroactive analysis for unknown drugs and increased confidence in automated processing capabilities, LC-HR-MS/MS has become a primary candidate for development of front-end comprehensive screening.

Objectives: The Georgia Bureau of Investigation currently performs front-end drug screening of whole blood by a combination of enzyme immunoassay (EIA) and liquid-chromatography tandem mass spectrometry (LC-MS/MS). EIA screens for opioids, cocaine, cannabinoids, barbiturates, and benzodiazepines. LC-MS/MS analyzes for 180 additional drugs. In an effort to streamline this process and make it more efficient, the creation and validation of a robust method utilizing a Q Exactive Focus high-resolution accurate mass spectrometer was investigated.

Methods: A 0.5 mL aliquot of whole blood is precipitated in a test tube by adding 1 mL of a 75:25 (acetonitrile:acetone) solution while vortexing. After precipitation the samples rest for 5 minutes allowing any emulsions to form. The samples are then vortexed to mix (breaking up any emulsions) and centrifuged for 10 minutes. The supernatant is poured over into a clean test tube fitted with a filtering reservoir. The filtering reservoir is removed, and the supernatant is split into two separate fractions for LC-HR-MS/MS analysis. A 0.1mL aliquot of the precipitate is taken and placed in a test tube and combined with 20 µL of 2% HCl in methanol and dried at 60°C for 3 min., then reconstituted with an 80:20 (H₂O:methanol) solution for a broad positive mode analysis method that looks for 184 analytes including opioids, anxiolytics, antidepressants, anti-psychotics, stimulants, and hallucinogens. The remaining precipitate is then combined with 2 mL of 0.1N HCl and 3mL of hexanes, vortexed for 30 seconds, and centrifuged for 5 minutes. The hexane layer is transferred to a clean test tube and evaporated to dryness for 5 minutes at 60°C. These samples are reconstituted in a 25:75 (H₂O: methanol) solution for negative/positive switching mode analysis that looks for barbiturates as well as THC and its metabolites.

Separation is conducted on an Agilent Poroshell 120 EC-C18 2.1x100 mm 2.7-micron column with a 4.5-minute gradient for the positive ion method at a 600 µL/min flow rate and over 8.5 minutes for the positive/negative ion switching method at a 200 µL/min flow rate. Mobile phases for both analysis consist of mobile phase A (0.1% formic acid and ~15 mM ammonium formate in water and mobile phase B (0.1% formic acid and ~15 mM ammonium formate in methanol:acetonitrile 50:50. Data acquisition software was set to produce a mass spectrum once a high resolution mass was detected in a retention time window of 0.2 min.

Results: A qualitative screen for all drugs of interest was developed and subsequently validated. Most drugs demonstrated little to no ion suppression producing LOIs of at least 2 µg/L for most drugs, with parent THC’s LOI being 0.5 ng/mL. Interferences of isomers were investigated; the findings demonstrated that although isomers are not always fully resolved, the instruments processing software can determine presence of multiple isomers with the exception of amitriptyline and matoxetine. A 1000 case concordance of postmortem and antemortem whole blood specimens demonstrated that the methods results were equivalent to the LC-MS/MS and EIA results traditionally obtained in case work.

Conclusion/Discussion: We successfully developed and validated a LC-HR-MS/MS method that would provide a more data rich sample set that is amendable to automated processing for the front-end screening of routine toxicology cases.
S42: Determination of 30 Synthetic Cathinones in Postmortem Blood using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

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Background/Introduction: Synthetic cathinones, commonly referred to as “bath salts,” are one of the top three new psychoactive substances (NPS) seized in the United States. They are powerful amphetamine-like psychostimulants that also share cocaine-like effects and have been sold as “legal highs.” Due to their desired effects, synthetic cathinones have increased in popularity worldwide since 2009. Although self-reported use of these substances has decreased in the past five years, they are increasingly used as adulterants in other recreational drugs such as ecstasy. Since illicit drug manufacturers constantly create new cathinones to evade judicial consequences, difficult challenges arise with the analysis and interpretation of such drugs. To keep up with these new stimulant drugs that continue to appear in the illicit market, up-to-date sensitive and robust methods are needed to reliably detect new cathinone analogs as they emerge.

Objectives: A low-sample-size liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed and validated to quantify 30 synthetic cathinones in postmortem blood with the application to selected casework specimens from 2015 to 2019 within the NYC Office of Chief Medical Examiner.

Methods: Solid phase extraction (SPE) using 0.25 mL postmortem blood was performed with mixed-mode cation exchange cartridges (Clean Screen® XCEL I, UCT Inc.). After reconstitution, all samples were separated in an Agilent Technologies 1600 Infinity LC equipped with Poroshell 120 EC-C18 column (2.1 mm x 100 mm, 2.7 µm) with 0.1% formic acid in water and 0.1% formic acid in acetonitrile as mobile phases. The total run time was 16 minutes and the flow rate was 0.4 mL/min. All analytes were detected by an Agilent Technologies 6460-triple quadrupole mass spectrometer operating electrospray ionization in positive mode. Two multiple reaction monitoring (MRM) transitions were monitored for each analyte. Ethylone-d5, mephedrone-d3, alpha-PVP-d8, dibutylone-d3 and methylone-d3 were used as internal standards. The method was validated in accordance to SWGTOX guidelines including bias, imprecision, matrix effect, extraction recovery, process efficiency, calibration model, carryover, interferences, stability and dilution integrity.

Results: The linear range of the calibration curve was 1 to 500 ng/mL (R^2 > 0.99) for all compounds with a weighting factor of 1/x (15 compounds) or 1/x^2 (15 compounds). Both imprecision and bias were evaluated at three different concentrations (3, 30 and 300 ng/mL) in triplicate each of the 5 days (n=15) of validation and met all allowed criteria (CV < 20%, bias 80-120%). No matrix effects were observed with values ranging from -5.1 to 13.3% (CV 11.4-17.5%, n=10). Extraction efficiency (84.9 to 91.5%) and process efficiency (86.1 to 102.6%) were satisfactory, except for 4-chloroethcathinone which was 63.0% and 64.9%, respectively. No carryover after the upper limit of quantification was detected (n=3). Neither endogenous (n=14) nor exogenous (amphetamines, cocaine, opioids) interferences were observed. This method was applied to 17 postmortem cases received between 2015 and 2019. Eight synthetic cathinones were detected: n-ethylpentylone (n=11, 19 to > 500 ng/mL), ethylone (n=3, 25 to > 500 ng/mL), butylone (n=2, 1.4 to 150 ng/mL), dibutylone (n=2, 13 to 486 ng/mL), methylone (n=1, 19 ng/mL), etylone (n=2, 1.6 to 2.5 ng/mL), 4-chloro-α-PVP (n=1, 7.2 ng/mL) and pentylone (n=1, 3 ng/mL).

Conclusion/Discussion: We developed a sensitive and specific liquid chromatography tandem mass spectrometry method for simultaneous determination of 30 synthetic cathinones in whole blood, employing 0.25 mL of matrix and achieving a 1 ng/mL limit of quantitation. To our knowledge, this method is the most comprehensive methodology for the determination of up-to-date synthetic cathinones currently available in whole blood. Eight different synthetic cathinones were detected in selected postmortem cases within the past four years, showing a wide range of concentrations from 1.4 to > 500 ng/mL.
Background/Introduction: Novel psychoactive substances (NPS) have steadily increased over the last decade. Within the United States, the most frequently encountered category of NPS has become synthetic opioids. Increased overdoses resulting from consumption of these new compounds has contributed to a national epidemic, with devastating public health impacts. Due to the rapid and unpredictable changes in availability of substances, similarity of chemical structures, specialized instrumentation required to detect sub-nanogram concentrations, and lack of reference standards, the prevalence of these substances is undoubtedly underreported in forensic casework.

Objectives: The objective of this presentation is to describe the analysis of previously acquired high resolution mass spectrometry (HRMS) data in order to retrospectively identify novel opioids including fentanyl analogs that were not included in the original scope of testing. The goals of this project included evaluating the time between when these NPS were being sold on the illicit market and when they were first identified in toxicology casework, as well as determining trends in prevalence.

Methods: Deidentified HRMS data files were archived from postmortem and DUID cases from our collaborating laboratory (NMS Labs, Horsham PA) between 2018 and 2019. The datafiles were retrospectively re-processed against frequently updated databases with new and emerging compounds. All analytical data was previously acquired on an Agilent 1290 liquid chromatograph coupled to an Agilent Jet Stream 6230 time of flight mass spectrometer (LC-TOF-MS). Agilent MassHunter Qualitative Analysis software was used for the identification of compounds. Standard reference materials were analyzed on the same platform to generate retention time and accurate mass data that was used for processing. The re-processing library contained over 170 different opioids and/or fentanyl analogs. Presumptive positive results were flagged using the following criteria: retention time ± 0.100 minutes relative to the library, the ppm error associated with the parent mass of less than 20, acceptable chromatography, the presence of correct isotopic pattern, and overall score. For positive findings of new substances that were not part of the original scope of testing, the data archive was retrospectively mined to determine date of first appearance.

Results: The preliminary results from January to December 2018 demonstrated a total of 12 new opioids or fentanyl analogs identified through this process (Table 1). Benzylfentanyl was identified a total of 9 times during this time period; however, it was identified by retrospective datamining in 3 cases before its inclusion in the out of scope findings in June 2018. Isopropyl U-47700 was identified in a total of 10 cases and was identified for the first time in May 2018 by the CFSRE. Retrospective datamining identified a further five cases between March and April 2018; however, it has not been detected again since October 2018. Phenylbenzylfentanyl was identified using datamining on two different occasions, once in February and one in June 2018.

Table 1. Datamining results for data acquired between January and December 2018

<table>
<thead>
<tr>
<th>Analyte name</th>
<th># Identified</th>
<th>Date of 1st detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isopropyl U-47700</td>
<td>10</td>
<td>Mar-18</td>
</tr>
<tr>
<td>Benzylfentanyl (R-4129)</td>
<td>9</td>
<td>Jan-18</td>
</tr>
<tr>
<td>Benzylfuranylfentanyl</td>
<td>9</td>
<td>May-18</td>
</tr>
<tr>
<td>Phenylfentanyl</td>
<td>5</td>
<td>Jan-18</td>
</tr>
<tr>
<td>3,4-Methylenedioxy U-47700</td>
<td>3</td>
<td>Jan-18</td>
</tr>
<tr>
<td>Alpha'-Hydroxyacetylfentanyl</td>
<td>2</td>
<td>Aug-18</td>
</tr>
<tr>
<td>Alpha-methylbutyrylfentanyl*</td>
<td>2</td>
<td>Jun-18</td>
</tr>
<tr>
<td>N-methylnorfentanyl</td>
<td>2</td>
<td>Sep-18</td>
</tr>
<tr>
<td>Ortho/Meta/Para-fluorofuranylfentanyl</td>
<td>2</td>
<td>Dec-18</td>
</tr>
<tr>
<td>Phenylbenzylfentanyl</td>
<td>2</td>
<td>Feb-18</td>
</tr>
<tr>
<td>4’/Para-Methylfentanyl</td>
<td>1</td>
<td>Apr-18</td>
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<tr>
<td>Despropionyl-ortho/3-methylfentanyl</td>
<td>1</td>
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</tr>
</tbody>
</table>

Conclusion/Discussion: The data shows that retrospective datamining can be a valuable tool to determine the prevalence and date of first appearance of novel compounds not contained within the initial scope of testing, without the need for re-extraction or retesting of the sample. Using datamining can help to evaluate the lag time between first detection and the incorporation of an analyte into the scope of testing to provide laboratories with an additional resource about how often scope updates are needed.

Keywords: LC-TOF-MS, Datamining, Novel Opioids
Background/Introduction: Electronic cigarettes (e-cigs) were developed as a method for nicotine delivery by the aerosolization of a liquid formulation made of a ratio of propylene glycol and vegetable glycerin and/or a pharmaceutical and/or herbal remedy plus, potentially, a flavoring agent. When the electronic cigarette is activated, the e-liquid is vaporized, followed by rapid condensation into an aerosol that the user inhales. E-cigs have been adopted by users to also inhale drugs other than nicotine (DOTNs), including cannabidiol (CBD). CBD is a significant active ingredient of *C. sativa* and *C. indica*. It has been purported to have anti-convulsant, anti-nociceptive, and anti-psychotic properties and is a popular ingredient in e-liquids. A single CBD formulation has been approved by the United States Food and Drug Administration for the treatment of two rare forms of epilepsy. The Farm Bill recently legalized hemp production in the United States, from which CBD can be extracted. If hemp contains more than 0.3% THC, the plant is considered a non-hemp product. A federally unregulated consumer-driven market of cannabinoid products, in combination with the loosely regulated e-cig market, has created public health and public safety challenges with toxicological emergencies.

Objectives: The purpose of this research was to identify unlabeled and unexpected psychoactive compounds in CBD e-liquids submitted by persons claiming unexpected untoward effects.

Methods: Eight samples were received from individuals across the United States who had purchased CBD e-liquid products from either online or retail outlets for therapeutic purposes. All samples were extracted into methanol and analyzed using a Shimadzu QP2020 GC-MS. In brief, an aliquot was injected onto a DB-5MS capillary column. The GC oven was programmed to an initial temperature of 70 °C followed by a 15 °C/min ramp to a final temperature of 300 °C held for 7 min with a total run time of 27 min. Analytes were identified using the SWGDRUG library and confirmed by comparing retention times and full scans using certified reference materials, where available.

Results: Seven of the eight samples contained CBD. The eighth sample contained MMB-FUBICA. The other 7 samples contained 5F-ADB, dextromethorphan, AMB-FUBINACA, or other cannabimimetic related compounds, including the JWH series. Other hemp related compounds were also identified.

Conclusion/Discussion: Most accounts were made by persons who claimed CBD use was for therapeutic benefit as opposed to recreational use. One account was described as having become addicted to CBD from recreational use. Other reports led to statements such as “I have not been able to leave my apartment for four days”, “What happened next I can only describe as the situation rapidly devolving into the scariest night of my life – I felt like I was dissociating from reality”, “one of the times I confronted [my son] in his room pale and glassy eyed”, and “a 79 year old grandmother just wanted to be pain-free, but she had severe hallucinations”. All persons were surprised by the effects but did not know what action could be taken to report the e-liquid companies or distributors. Several were concerned that any attention would have adverse recourse, both personally and professionally.

Analysis of the eight samples revealed a number of unexpected psychoactive compounds that accounted for the untoward effects the individuals described. The federally unregulated cannabinoid market, combined with an e-cig market with loose quality assurance requirements, has created a significant public health and public safety problem in the United States. As such, these cases highlight the need for the forensic toxicology community to publish findings from casework which could demonstrate the breadth of the problem and provide a warning with wider implications for public safety.
S45: Syncan or Syncan’t, There is No Try

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Background/Introduction: Over the past ten years, forensic toxicologists have been battling the emergence of synthetic cannabinoids from the original JWH series, to AM variants, from indoles to INACAs. This year, compounds incorporating facets from each of these groups were identified in human performance casework. A common feature among these cases involves vaporized products, particularly those disguised within the seemingly innocuous trend of cannabidiol (CBD) use.

Objectives: This presentation raises awareness about prominent synthetic cannabinoid derivatives in forensic casework this past year.

Methods: Blood and/or urine specimens were submitted to the Division of Forensic Toxicology as part of suspected driving while intoxicated or military fit-for-duty investigations. Routine screening encompassed a drugs of abuse immunoassay for 9 classes of drugs along with an alcohol screen by headspace gas chromatography. If case history indicated potential CBD use, a CBD-specific liquid chromatography tandem mass spectrometry (LC-MS/MS) method was utilized as a sensitive and specific screening technique. If screens were negative, testing escalated to include liquid chromatography time of flight mass spectrometry (LC-QTOF/MS) with 5F-ADB, MMB-FUBICA, 5F-MDMB-PICA, AB-FUBINACA, FUB-AMB, along with their respective acid metabolites included within the library. Presumptive screens were confirmed by LC-MS/MS analysis, with a limit of detection at 0.1 ng/mL.

Results: Since late March 2018, our laboratory has identified parent drug along with metabolites in approximately 100 cases containing either single or multiple cannabinoids. In one incident, a 21-year-old male seemed to be behaving erratically. On multiple occasions, he had slurred speech and vomited. Confirmatory analysis of the urine specimen revealed the presence of 4F-MDMB-BUTINACA 3,3-dimethylbutanoic acid.

A second incident involved a young male who missed morning formation. When others went to check on him, he was found to be sluggish and his room smelled like a scented vapor. AB-FUBINACA and FUB-AMB 3-methylbutanoic acid (AB-FUBINACA metabolite 3) were confirmed in urine.

A third service member admitted to smoking CBD oil from a vape shop. The CBD analysis was negative, but FUB-AMB 3-methylbutanoic acid was confirmed instead. His companion also had FUB-AMB 3-methylbutanoic acid in urine, but 4F-MDMB-BUTINACA 3,3-dimethylbutanoic acid was also present.

In another case, security forces encountered a young male who was acting strangely and under the influence of an unknown substance. It was his third instance in the last three days where he was incoherent and lethargic. Alcohol, immunoassay, and CBD were negative, but 4F-MDMB-BUTINACA 3,3-dimethylbutanoic acid was subsequently confirmed by LC-MS/MS.

Conclusion/Discussion: Although it is difficult for most forensic laboratories to keep up with the changing landscape of designer drugs, case history and time of flight mass spectrometry is vital to identifying other potential substances that may be otherwise overlooked. In addition, CBD vaping liquids were previously described in scientific literature to contain synthetic cannabinoids, as well as other substances with potential impairing effects. These results demonstrate how important it is to stay abreast of current trends, frequently update methods with relevant synthetic cannabinoid compounds, and conduct synthetic cannabinoid analyses as part of routine forensic toxicology casework.

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2National Institute for Health and Welfare, Forensic Toxicology Unit, Helsinki, Finland

**Introduction & objectives:** The appearance of new psychoactive substances (NPS) in the illicit drug market poses demanding challenges to the healthcare, law enforcement, and forensic toxicology laboratories. Focusing on the latter, we have worked on a method that is designed to tackle a common analytical problem related to NPS—the unavailability of authentic reference standards.

**Methods:** We introduce a new analytical platform in NPS bioanalysis which consists of gas chromatography (GC) coupled to a nitrogen chemiluminescence detector (NCD) and quadrupole time-of-flight mass spectrometer (QTOFMS), interfaced with atmospheric pressure chemical ionization (APCI) source. A two-way splitter directs the GC flow simultaneously to APCI/QTOFMS for tentative identification and to NCD for universal quantification. Chromatographic peaks observed in both detectors are aligned to elucidate the identity of the quantified peaks in the NCD chromatogram.

The universal quantification by NCD is based on the linear and equimolar response to nitrogen. Therefore, all nitrogen-containing drugs (approximately 90% of all drugs) are within the scope of this method. In our previous studies, we have concluded that GC coupling provides better sensitivity than liquid chromatography in quantitative NCD measurements, which is desirable in bioanalysis. To control quantitative analysis, three external calibrators were selected to cover for prim, sec and tert- amines (amphetamine, methamphetamine and MDPV, respectively).

For identification, we selected APCI as an ion source for GC-QTOFMS since the preservation of the protonated precursor ion is required for accurate mass-based tentative identification (± 2 mDa threshold). Using a data independent acquisition mode in GC-APCI-QTOFMS, the precursor and qualifier ion data were acquired without any preselection of the target ions.

We applied the GC-NCD-APCI-QTOFMS platform to study 38 illicit psychostimulants, with emphasis on NPS, in spiked post-mortem blood. The post-mortem blood samples were extracted with butyl chloride/ethyl acetate (3:1 v/v) at a basic pH followed by acylation with trifluoroacetic anhydride. In addition, eleven post-mortem blood samples obtained from routine casework were analyzed by the GC-NCD-APCI/QTOFMS method and the results were compared with an established electron ionization GC-MS method with appropriate calibration.

**Results:** In total, 35 out of 38 spiked psychostimulants were successfully quantified with a limit of quantification (LOQ) of 0.05 mg/L. The between-day accuracy was 62.3 – 143.3% (mean 93.5%, median 88.5%) and precision was 6.6 - 22.4% CV (mean 15.8%, median 16.1%). Unfortunately, we were unable to quantify dibutylone, MDBD and methylphenidate at low levels because a commonly occurring matrix components were obscuring the chromatographic peaks in the GC-NCD. The agreement between electron ionization GC-MS (with reference standards) and GC-NCD-APCI-QTOFMS (without reference standards) in eleven post-mortem blood samples containing 0.08 – 2.4 mg/L of amphetamine (n = 5), methamphetamine (n = 4) or MDMA (n = 4) was 62.3 – 117.3%.

**Conclusion:** This is the first study to apply the recently introduced GC-NCD-APCI/QTOFMS platform to the accurate mass-based tentative identification and quantitative estimation of drugs in human blood, simulating an analysis where no authentic reference standards were available. With this approach, identification and quantification can be confirmed retrospectively for cases where the structure of the NPS was initially unknown. We conclude that, from the limited number of techniques available within analytical toxicology, GC-NCD-APCI/QTOFMS is among the most viable approaches to instantly estimate concentrations of stimulant NPS in blood.
Background/Introduction: Oral fluid has become an increasingly popular biological sample for drug testing in the investigation of driving under the influence of drugs (DUID) cases. Performing oral fluid drug testing at the point of contact (POC) is an especially useful tool for producing timely investigative information and supporting officer suspicions about drug use immediately in the field. This approach may facilitate police investigations, provide actionable drug use information about suspects, and save time and resources in the field.

Objectives: The objective of this research was to evaluate the performance of the Securtec DrugWipe® S 5-Panel against its claimed scope and analytical sensitivity. Specifically, the evaluation was designed to: 1) assess the device performance relative to the manufacturer’s published cutoffs; 2) assess the ability to produce positive results in polydrug cases; 3) investigate the cross-reactivity of commonly encountered drugs, and metabolites and; 4) evaluate the effects of potential interferences (e.g. oral hygiene products, beverages and tobacco) on the device performance.

Methods: All testing was performed using authentic drug free, pooled oral fluid (30µL). Cutoff evaluations were performed at the cutoff (Table 1) and ±50% of the cutoff concentration in replicates of ten. The second part of the cutoff evaluation consisted of running a series of mixed drug controls containing the target analytes at various concentrations. Cross-reactivity was assessed for in triplicate for 24 commonly encountered drug metabolites, therapeutic drugs and other drugs known to cross-react on immuno-assay tests and miscellaneous other drugs at a concentration of 1000 ng/mL. Commonly encountered potential interferences (e.g. coffee, mouthwash, alcohol, tobacco) were assessed in a series of experiments to assess matrix effects and signal suppression. All results were recorded using an instrumented reader (WipeAlyser®). Results were scored as true positives (TP) if the analyte was present in the aliquot and detected by the device, irrespective of its concentration. With all negative results, the concentration of each drug in the aliquot was compared to the manufacturer’s specified cutoff concentration. All results were processed using receiver operator characteristics (ROC) analysis.

Table 1.

<table>
<thead>
<tr>
<th>Drug Assay</th>
<th>DrugWipe 5S Cutoff (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>THC</td>
<td>5</td>
</tr>
<tr>
<td>Cocaine</td>
<td>10</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>80†</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>80†</td>
</tr>
<tr>
<td>Opiates (morphine)</td>
<td>10</td>
</tr>
</tbody>
</table>

†DrugWipe has a combined amphetamine/methamphetamine panel.

Cutoffs reflect parent drug only.

Results: The overall sensitivity for the cutoff evaluations was 100% for THC, cocaine, amphetamine, and methamphetamine and 80% for opiates. The WipeAlyser® detected seven THC positive results, five morphine positive results, seven amphetamine and eight methamphetamine positive results at 50% below the cutoff concentration. With respect to cross-reactivity, the WipeAlyser® detected both MDMA and MDA at 100 ng/mL, benzoylecgonine at 500 ng/mL, codeine at 10 ng/mL, both hydromorphone and hydrocodone at 100 ng/mL, 11-nor-delta-9-THC-COOH was detected at 10 ng/mL and cannabinol at 100 ng/mL. The consumption of gum immediately prior to the test produced two false negative results for opiates, while tobacco consumption yielded four false positives; two on the amphetamines panel and two on the opiates.

Conclusion/Discussion: The DrugWipe 5S® has a relevant test panel, testing for the most commonly encountered illicit drugs in drivers: THC, amphetamine/methamphetamine, cocaine and opiates The WipeAlyser® detected seven THC positive results, five morphine positive results, seven amphetamine and eight methamphetamine at 50% below the cutoff concentration. Recommendations related to device performance specifications have been previously described in the ROSITA and DRUID projects. The ROSITA project recommended greater than 95% accuracy compared to 80% recommended by the DRUID project. When evaluating the cutoff results, the Drug Wipe 5S meets both sets of accuracy recommendations for all assays when using the WipeAlyser® analyzer. No false positive results were observed during the testing cutoff, mixed drug or cross-reactivity assessments.
S48: Illicit Drugs Trends in UDT by Age Groups in Pain Management Population

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**Background/Introduction:** Monitoring drugs of abuse in the pain management population is critical due to their strong addictive potential and interference with narcotic treatment of pain patients, which can lead to potential discontinuation of the treatment. Physicians determine patient medication compliance by ordering individualized urine drug testing (UDT). Patient specific UDT order becomes a challenge when physicians need to decide whom to test and what tests should be ordered. Ordering illicit drug tests in conjunction with justification of medical necessity is not always a straightforward decision because patient’s age, history of relationship with physician, and risk assessment are some of the factors that play a role in ordering UDT. It is a common belief that middle-aged to older population is less predisposed to the use of illicit drugs, and therefore not tested for their presence.

**Objectives:** To evaluate the prevalence of illicit drug use within the pain management population. Understanding the extent of illicit drug use in the middle-aged to older population to assist physicians in justifying the needs of ordering illicit drug tests as part of compliance, medical necessity and therapeutic monitoring.

**Methods:** National Spine and Pain Centers, LLC (NSPC) conducted a retrospective analysis of both prescription and illicit urine drug test results from 2014 to 2018. Tests were ordered for patients by pain management healthcare providers in order to determine compliance and therapeutic monitoring. All positive illicit drug results were reviewed and classified into groups based on age and sex of the patients. Heroin and cocaine were determined based on the presence of their metabolites 6-acetylmorphine and benzoylecgonine, respectively. Ecstasy and PCP were identified by parent drug presence. Illicit D-methamphetamine isomer was distinguished from L-methamphetamine isomer (from Vicks Vapor Inhaler), whileamphetamine was included if it was not prescribed.

**Results:** This study included review of 300,000 UDT reports for patients of 45 years and older (male (41%)/ female (59%)), which would represent 82.3% percent of all pain population in NSPC clinics.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Heroin metabolite (6-AM)</th>
<th>Cocaine metabolite (Benzoylecgonine)</th>
<th>Ecstasy (MDMA/MDA)</th>
<th>Methamphetamine</th>
<th>PCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>45-54</td>
<td>0.50%</td>
<td>3.71%</td>
<td>0.01%</td>
<td>0.15%</td>
<td>0.13%</td>
</tr>
<tr>
<td>55-64</td>
<td>0.30%</td>
<td>2.50%</td>
<td>0.00%</td>
<td>0.12%</td>
<td>0.10%</td>
</tr>
<tr>
<td>65-74</td>
<td>0.12%</td>
<td>0.98%</td>
<td>0.00%</td>
<td>0.02%</td>
<td>0.02%</td>
</tr>
<tr>
<td>Over 75</td>
<td>0.00%</td>
<td>0.35%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.02%</td>
</tr>
</tbody>
</table>

Cocaine was found to be the most prevalent drug of abuse in all groups of middle age to older population. Ecstasy was the rarely used in middle-aged to older population. Heroin, cocaine and PCP were about two times more prevalent in males, while amphetamine was about two times more prevalent in females. Methamphetamine was equivalently found in both sex groups. Our research found the majority of the drugs of abuse were found in conjunction with prescribed medications – opioids, benzodiazepines, skeletal muscle relaxants, or even with several combinations of illicit drugs. Amphetamine and cocaine, cocaine and heroin (Speedball) are the top illicit drug combinations.

**Conclusion/Discussion:** Cocaine can be recommended for testing more frequently in middle-aged to older population, while ecstasy is rare in the same age group. The pain population is very vulnerable to abusing illicit drugs and their use is often underestimated and underdiagnosed. This study and future studies will help physicians to justify their UDT orders of illicit drugs in pain management practices.
S49: Implementation of a Blind Quality Control Program in Blood Alcohol Analysis

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Background/Introduction: Declared proficiency tests (PTs) are beneficial to laboratories to measure results and processes against other laboratories in the country but are typically completed once per year per analyst as part of the accreditation requirement. Declared PTs are limited in their use for testing the performance of the entire system because analysts are aware that they are being tested. A blind quality control (BQC) sample is intended to appear as a real case to the analyst to remove any intentional or subconscious bias. By removing bias, laboratories can more effectively and realistically gauge the performance of their processes.

Objectives: To provide continuous quality improvement and a constant and real-time assessment of the laboratory processes, the Houston Forensic Science Center (HFSC) incorporated a BQC program in blood alcohol analysis in September 2015 as a supplement to declared PT tests. It allows HFSC to monitor the reliability of procedures used in casework from evidence intake to reporting.

Methods: Between September 2015 and July 2018, HFSC submitted 317 blind cases: 89 negative samples and 228 positive samples at five target concentrations (0.08, 0.15, 0.16, 0.20, and 0.25 g/100 mL; theoretical targets). For the blind samples to mimic real toxicology cases, the case information and its associated paperwork were fabricated and placed, along with the blood tubes, into typical collection kits used by the submitting agency. These BQC cases were submitted, received, and analyzed by HFSC in the same manner as real evidence. These samples were analyzed by a headspace gas chromatograph interfaced with dual flame ionization detectors (HS-GC-FID); the limit of quantification for ethanol was 0.01 g/100 mL.

Results: All negative samples produced “no ethanol detected” results. The mean (range) of reported blood alcohol concentrations (BAC) for the aforementioned target concentrations was 0.075 (0.073-0.078), 0.144 (0.140-0.148), 0.157 (0.155-0.160), 0.195 (0.192-0.200), and 0.249 (0.242-0.258) g/100 mL, respectively. The average BAC percent differences from the target for the positive blind cases ranged -0.4 to -6.3%, within our uncertainty of measurement (8.95-9.18%). The rate of alcohol evaporation/degradation was determined negligible. A multiple linear regression analysis was performed to compare the % difference in BAC among 5 target concentrations, 8 analysts, 3 HS-GC-FID instruments, and 2 pipettes. The variables other than target concentrations showed no significant difference (p-values >0.2). While the 0.08 g/100 mL target showed a significantly larger % difference than higher target concentrations (0.15-0.25 g/100 mL), the % differences among the higher targets were not concentration-dependent. This suggested that the source of the theoretical target level variability was likely from the manufacturing process rather than the systemic bias from the analytical method.

Conclusion/Discussion: Despite difficulties like gaining buy-in from stakeholders and mimicking evidence samples, the implementation of a BQC program has improved processes, shown methods are reliable, and added confidence to staff’s testimony in court. The 2017 revision of the General requirements for the competence of testing and calibration laboratories standard published by the International Organization for Standardization/International Electrotechnical Commission (ISO/IEC 17025) requires laboratories to monitor the validity of their results. Clause 7.7.1 lists out several quality controls that a laboratory may implement to conform to this requirement, and the BQC program fulfills both 7.7.1 j) intralaboratory comparisons and 7.7.1 k) the testing of blind samples. HFSC has yet to receive an unsatisfactory result from a blood alcohol analysis BQC case. The results indicate no significant variation in blood alcohol results over time, between analyst, instrument, or pipette. Therefore, we can conclude that our methods are reliable and produce accurate results. HFSC encourages other forensic laboratories to consider implementing their own BQC program that is tailored to their specific needs as a reliable means for evaluating their processes and complying with accreditation requirements.
SSO: Endogenous GHB in Hair: A Large Population Study

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Background/Introduction: Drug-facilitated crimes (DFC) can be difficult to corroborate with traditional toxicology samples like blood or urine due to reporting delays. Hair can often be a useful sample addition for testing in DFC cases as it provides a longer detection window (1-3). Delay in reporting a DFC that involves GHB (gamma-hydroxybutyric acid) is also complicated by its rapid metabolism and clearance from the body, as well as its endogenous presence. While many studies over the years have worked to resolve the challenges encountered when analyzing GHB in hair, few have had large populations. Additionally, new statistical approaches for demographic hair comparison and differentiation of endogenous and exogenous GHB are discussed.

Objectives: By testing a large population of non-GHB users, the audience will be able to understand and draw conclusions about endogenous GHB concentrations with the goal to better differentiate endogenous from exogenous GHB in hair.

Methods: Hair samples and demographic information were collected with documented informed consent from 214 donors. The hair samples were cut close to the scalp, segmented into 1 cm portions, decontaminated, and ground to a fine powder. A 10 mg sample of each segment was processed by 1 M NaOH base digestion, ethyl acetate liquid-liquid extraction, and liquid chromatography tandem mass spectrometry analysis. An Acclaim® Trinity™ P1 column (2.1 mm × 150 mm, 3 μm) with guard column (2.1 mm × 10 mm, 3 μm) was used. Mobile phase A contained 45% 25 mM ammonium acetate at pH 5.49 and 55% acetonitrile; mobile phase B was 100% water (Optima LC-MS grade).

Estimations of GHB in the donor samples were completed using a calibration curve prepared by spiking GHB into SMx™ Hair liquid matrix (UTAK®, Valencia, CA) from 0.4-12.0 ng/mg. The quantitative data were analyzed using Minitab™ and Excel™ software packages along with bootstrapping and Kruskal-Wallis statistical tests.

Results: A total of 2074 hair sample segments from 141 women and 73 men (all collected hair 3-12 cm in length) were analyzed. The range of endogenous GHB concentrations observed was <0.4-5.5 ng/mg and 97.5% of the segmental results were less than 2 ng/mg. Statistical analysis was performed on a segmental basis and the results (e.g., median, etc.) will be discussed. A Kruskal-Wallis comparison of segmental medians in males and females indicates that these groups are different, with greater than 95% confidence. Samples from 73 female donors in this study experienced some type of chemical and/or thermal treatment within the year prior to collection. Comparing “treated” to “untreated” hair in the female group led to subpopulation differences, with greater than 95% confidence. Age groups and races were also analyzed for differences, but none were significant at α=0.05.

Female hair samples appeared to have a trend comprising higher endogenous GHB concentrations close to the scalp and with a net decrease of ~0.2-0.3 ng/mg distally. Male hair samples displayed the opposite trend, with a net increase of ~0.5-0.6 ng/mg from the proximal to the distal end of the hair shaft. However, there was minimal change between individual adjacent hair segments, with 97.1% of adjacent segment differences within ±0.5 ng/mg, across the population.

Conclusion/Discussion: This is the largest hair population of non-GHB users studied to date. The endogenous GHB concentration range was largely consistent with previously published data (4). Based on the wide concentration range detected in our population, it appears difficult to select an appropriate cut-off for differentiating endogenous GHB from exogenous without a large controlled dosing study. There was differentiation based on gender and chemical/thermal treatment. Other variables did not significantly differentiate subpopulations. Additionally, using adjacent segment concentration differences could be a strategy to assist in differentiating endogenous from exogenous GHB exposure.

References:

Background/Introduction: In France, 600,000 elderly people are in accommodation facilities for dependent people and more than half have cognitive disorders or dementia.

Objectives: To identify the cause of the disappearance of some psychotropic drugs in the pharmacy of a Geriatric Long-Term Care Unit.

Methods: Many cases of missing drugs were reported by the executive nurse in a geriatric Long-Term Care unit. Different psychoactive drugs were implicated such as midazolam while no patients had such a prescription. Four hypotheses have been proposed: 1. stolen by a nurse for his own addiction, 2. stolen by a nurse in order to resale, 3. mistaken in traceability of inventory movements of the drugs, and 4. misused of the drugs without prescription by a nurse to calm the restless patients during the night. In order to exclude mistreatment, blood and hair samples were successively analyzed without the knowledge of the staff early in the morning for 5 patients with the most frequent agitation crises. When hair was sampled, a 2-cm segmental analysis was performed when possible (length ≥3cm). TSQ Endura LC/MS/MS targeted screening and quantitation (Thermo Fisher®) were performed in blood or a 20 mg-hair sample after decontamination and Liquid/Liquid extraction in basic conditions. The method of quantification was accredited and included all psychoactive drugs such as drugs-of-abuse, neuroleptics, hypnotics, antidepressants, anxiolytics, anti-epileptics, and other sedatives. Limits of quantification were between 1 and 10 ng/mL or pg/mg according to the compounds.

Results: In the first blood analysis, one of the patients was found positive for midazolam (0.9 ng/mL) and OH-midazolam (0.6 ng/mL). This patient initially tested negative with a TSQ Access MAX triple quadrupole method (Thermo Fisher®), a less sensitive instrument compared to the TSQ Endura, showing that instrument sensitivity is critical in suspected mistreatment cases. This result indicated the administration of a non-prescribed compound in this patient. Hair testing performed in 5 additional patients showed the presence of non-prescribed midazolam. The concentrations were low in 4 of the patients (1-12 pg/mg) in accordance with single administration and was found higher in the last patient (40-120 pg/mg in the different segments). Additional non-prescribed therapeutic drugs were also identified in these 5 patients’ hair: zolpidem (4 patients), carbamazepine (4), clobazam (3), tramadol (2), nefopam (1), lamotrigine (1), zopiclone (1), alprazolam (1), levetiracetam (1), citalopram (1), venlafaxine (1), and codeine (1). Each patient tested positive for 2 to 9 non-prescribed compounds in their hair. After implementing modified security and delivery of the pharmaceuticals in the unit, blood samples were analyzed 3 months later. These results revealed that 3 patients were still positive for a non-prescribed medicine, 1 for zopiclone (while this substance was no longer available in the unit), 1 for clobazam (not available), and 1 for paroxetine (still available for the nurse staff). The corresponding hair analysis performed simultaneously on the 5 same patients showed the disappearance of all the benzodiazepines and “Z drugs” (midazolam, alprazolam, zolpidem, zopiclone) except clobazam still present in 2 patients (concentrations decreased relative to initial test results). Carbamazepine (4), lamotrigine (1), and levetiracetam (1) were still present and two new antidepressants appeared in patients at low, possible single dose levels, mirtazapine (2) and venlafaxine (1). These low positives could be due to a mistakenly and unintentionally administration since different patients in the same room received these treatments as those that erroneously tested positive. After reporting to the prosecutor, a press conference by the General Manager of the “Assistance Publique - Hôpitaux de Paris” led to the secure delivery of all psychotropic drugs in all the geriatric departments of our institution and suspension of a nurse suspected of improperly administering medications. In order to confirm the disappearance of mistreatment, 19 patients’ hair including the 5 initial patients were collected 6 months later and confirmed the absence of non-prescribed psychoactive substances in the 1 or 2-cm proximal hair segment.

Conclusion/Discussion: This study shows the need for a sensitive toxicological analysis and the reliability of hair testing in suspected mistreatment cases of hospitalized elderly people. Interpretation of results from blood and hair needs to be delicately considered as there is the possibility of accidental drug administration mistakes due to multiple drugs for patients in shared rooms.
Background/Introduction: It is not unusual that a victim suspects they were drugged in connection with a sexual assault or other violent crime. However, earlier studies from our laboratory have shown that the presence of other substances than ethanol in victims is scarce and concluded that drug facilitated sexual assaults are rare. The fact that more than half of the victims had ethanol on board pointed towards that as the primary cause of impairment. During 2011, the National Board of Forensic Medicine introduced a routine drug and medication screening using high resolution mass spectrometry in all violent crimes, increasing the chances to detect substances commonly used in drug facilitated crimes.

Objectives: The aims of our study were to describe the toxicological findings in cases of violent crimes and compare the results from cases where the victim reported a suspected drugging or when they did not.

Methods: Retrospectively, we retrieved information from the National Board of Forensic Medicine’s case database. All cases from victims of suspected violent crimes between 2012 and 2018 were identified. Cases were the suspected offense included “rape”, “sexual assault”, “spiking”, or “drugging” were then selected. Based on the police request the cases were divided into two groups, no suspected drugging and DFC.

Results: There was a total of 3903 cases of violent crime where 2813 were classified as sexual assaults or druggings. Because of lack of background data 344 could not be grouped resulting in a study population consisting of 1100 DFC cases and 1369 cases where no drugging was suspected.

The mean age was 25.0 and 25.4 in the DFC and non-DFC groups. In 32.8 % of the DFC cases the toxicological analyses were completely negative as compared to 30.3 % in the non-DFC group. Ethanol detection rate was significantly different between the two groups with 48% of the cases with no suspicion of drugging and 41% in the DFC group. The mean blood alcohol concentration was 1.14 promille (0.12 g/dL) and 1.06 promille (0.11 g/dL), respectively with no significant difference between the two groups. In DFC cases 17% had one or more hypnotic or sedative drug positive whereas in the other group only 11% were positive suggesting an overrepresentation in DFC cases. The same trend was seen for central stimulants (12.4% vs 7.5 %), opioids (7.7% vs 5.8%), and cannabis (10.3% vs 6.6%). Other medications, on the other hand, was more prevalent in the non-DFC group with 23.5% positives compared to 12.3%. The most common sedatives found in blood were diazepam, alprazolam, and clonazepam. The concentrations were within the therapeutic ranges and did not differ between groups. However, therapeutic concentrations do not preclude impairment, especially not in naive users or when combined with alcohol.

Conclusion/Discussion: In conclusion, there were significant differences in findings between DFC cases and cases where no drugging was suspected. We found a lower proportion of ethanol in the DFC group but the mean BAC was similar. DFC cases had a higher proportion of abused drugs and sedatives present. Indeed, one out of six of the DFC cases had a sedative on board pointing towards a potential drugging.