K1 Drivers Under the Influence of Alcohol and Drugs: An Eight-Year Retrospective Analysis in a Southern Italian Region

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After attending this presentation, attendees will better understand the Italian trend of alcohol and drug use among motor vehicle drivers involved in Road Traffic Crashes (RTC).

This presentation will impact the forensic science community by providing alcohol and drug results of biological samples collected from 1,797 drivers at the request of police from 2009 to 2016 that were processed at a major forensic toxicological laboratory in southern Italy.

Operating a motor vehicle while Driving Under the Influence Of Alcohol (DUIA) or Drugs (DUID) is considered a crime worldwide because of the risk to traffic safety. Based on the recent report by the National Institute for Statistics (ISTAT) in Italy, there were 173,892 traffic accidents resulting in personal injury in 2015. From 2013 to 2015, an average of four to six deaths and 20 injured drivers per 100,000 people were recorded by ISTAT in the Campania region, the third most-populous region in Italy. This region has a population of 5,869,965 people, with 4,434,136 inhabitants living in the Naples metropolitan area alone, the second most-populated metropolitan area in Italy, after Milan.

A recent Italian Road Traffic Law (IRTL) (L. 41/2016) just updated the crimes related to DUIA and DUID with the penal sanctions having been generally increased. If a driver causes the death of one person and injury to another, he can be punished with 18 years in prison and at least 5 years disqualification from driving. In the Campania region, the Forensic Toxicology Unit (FTU) of the University "Luigi Vanvitelli" of Campania represents the "reference laboratory" of the entire region, performing all of the confirmation toxicological analyses for medicolegal purposes. The toxicology lab is accredited to perform the analytical work on postmortem samples as well as on hospitalized drivers injured because of RTC. According to the sampling protocol established by the current IRTL, when drivers are injured in an RTC, a medical evaluation must be performed first. Immunochemical screening tests on biological samples must follow in order to find evidence of alcohol/drug effects on the driver's performance. Only positive blood and urine samples collected from injured drivers are forwarded to the FTU for confirmation of the toxicological analyses.

To assess the trends in the use of alcohol and drugs among motor vehicle drivers, a retrospective analysis was performed based on drivers involved in RTC and admitted to 16 Emergency Departments (ED) located in the different provinces of the Campania region from 2009 to 2016. An additional goal of the study was to collect data useful to the improvement of toxicological analytical work and preventive policies with regional relevance. Confirmation tests of positive toxicological screening analyses were performed on biological samples (blood/urine) collected from 1,797 hospitalized drivers. The analyses were performed on a total of 780 blood samples: 609 cases were referred for suspected DUIA and 171 cases for suspected DUIA and DUID; 1,017 urine samples were also collected from DUID cases when the blood test was denied by drivers. All blood and urine samples were collected at admission to the ED within two hours of the accident. Blood Alcohol Concentration (BAC) on whole blood was analyzed by Headspace/Gas Chromatograph/Flame Ionization Detector (HS/GC/FID). Qualitative and quantitative analyses for drugs were accomplished by Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).

Results: BAC greater than 0.5g/L (the legal limit in Italy) was observed in 91.5% of drivers suspected for DUIA and in 93% of drivers suspected for DUID. In particular, BAC >1.5g/L were found in 308 suspected cases of DUIA out of 609 (50.5%) and in 66 suspected cases of DUID out of 171 (38.6%). RCT occurred mostly in drivers with BAC >1.5g/L, while in cases of DUID, BACs between 0.5g/L and 1.5g/L were most common. Toxicological analyses for drugs in blood were negative in 51 drivers out of 171 DUIA and DUID cases total (29.8%). Cocaine and Δ^9-Tetrahydrocannabinol (Δ^9THC) were the drugs most commonly associated with alcohol, followed by poly-drug abuse, a combination of different drugs among which, again, cocaine and THC were the most represented, followed by methadone and Benzodiazepine (BDZ). Among positive urine analyses, 11-nor-9-Carboxy-Δ^9-Tetrahydrocannnabinol (THCCOOH) was the most frequently identified compound, alone or in association with other drugs, followed by poly-drug>cocaine>BDZ>opiates. It is worth mentioning that negative confirmation tests were obtained in 14.5% of the drivers previously recognized as positive in screening analyses. Therefore, an improvement in the protocols currently applied to DUIA and DUID assessment is needed, and confirmation tests on the blood should be considered mandatory in demonstrating a violation of the Road Traffic Act.

Driving Under the Influence, Blood Alcohol Concentration, Road Traffic Crashes

K2 Surface-Enhanced Raman Spectroscopy (SERS) -Based Screening Test for Synthetic Cannabinoids in Oral Fluid

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After attending this presentation, attendees will better understand SERS and how it can be used to retrieve structural information from small molecules at very low concentrations. Attendees will also understand how this approach can be applied to the detection of xenobiotics in oral fluid.

This presentation will impact the forensic science community by demonstrating how SERS is a fast, selective, and sensitive approach for synthetic cannabinoids screening, as an alternative to immunoassays.

Synthetic cannabinoids are New Psychoactive Substances (NPS) that represent a worldwide issue due to unknown toxicological effects and widespread use among the young population. Moreover, the rapidity by which these compounds are modified and introduced into the illegal market makes it difficult to detect them using standard screening methods, such as immunoassays. Indeed, cross-reactivity between different species and false negative responses severely limit this approach.

SERS has been shown by this study to have great potential to solve these problems by providing a sensitive and selective screening approach that can provide fingerprint signals from xenobiotics at toxicological concentrations. This was achieved on benzodiazepines, both as standard solutions and in spiked urine matrices, and more recently, on standard solutions of synthetic cannabinoids. The latter included JWH-018, JWH-030, JWH-073, JWH-081, JWH-122, JWH-175, AM-2201, MAM-2201, with typical Limits of Detection (LODs) ranging from 20ng/mL for JWH-018 to 140ng/mL for JWH-081 and AM-2201. By translating this strategy to biological matrix analysis, the forensic and emergency medical field would benefit from a sensitive and selective alternative to current immunoassays. Because the procedure provides a spectral fingerprint, SERS can be seen as a complementary tool to other structure elucidation techniques, such as mass spectrometry.

SERS is a surface spectroscopy that amplifies Raman scattering by several orders of magnitude via the addition of metallic nanoparticles capable of producing Localized Surface Plasmon Resonance (LSPR). In this method, citrate-reduced gold nanospheres were prepared as LSPR-bearing substrates and later aggregated through the addition of $MgCl_2$. The aggregation process red-shifts the frequency of the LSPR and produces strong electromagnetic fields where the particles interact. The result is a rapid method for detection with exceptional sensitivity.

This presentation will focus on the development of an optimal extraction technique to detect synthetic cannabinoids in oral fluids. Fortified oral fluid samples were pretreated via centrifugation in the presence of methanol, which yielded protein sedimentation. Throughout the course of this work, thiocyanate anions were found to be a critical interfering species, as they strongly interact with the colloidal gold. Therefore, a variety of different desalting procedures were examined prior to SERS analysis, including ion exchange and solid phase extraction. The SERS signal was also increased through various wash steps conducted on the gold colloid, prior to its use as an enhancing substrate. This reduced residual citrate molecules carried over from the synthetic process, leaving the nanoparticle's surface more available for analyte adsorption.

The optimized methodology was developed using JWH-018 as a model target drug, then extended to other naphthoylindole synthetic cannabinoids. Detection was achieved using a portable Raman spectrometer operating at 785nm. This new procedure has great potential in forensic analysis, both as a more specific and flexible replacement for immunoassay and as an orthogonal method for analysis that is compatible with downstream mass spectral detection.

Major support for this research has been provided by an award from the National Institute of Justice. Points of view in the document are those of the authors and do not necessarily represent the official view of the United States Department of Justice.

SERS, Synthetic Cannabinoids, Oral Fluid

K3 Will the Real "Molly" Please Stand Up? N-Ethyl Pentylone-Related Deaths in Alabama

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The goals of this presentation are to: (1) review n-ethyl pentylone-proposed pharmacology; (2) describe the n-ethyl pentylone case facts; and, (3) recognize the increasing prevalence of n-ethyl pentylone and the potential trend of combining it with cocaine.

This presentation will impact the forensic science community by increasing knowledge and awareness of n-ethyl pentylone through communication of its increasing prevalence as observed in the Birmingham, AL, area and highlighting the potential new drug trend of combining n-ethyl pentylone and cocaine.

Hypothesis: N-ethyl pentylone, a Novel Psychoactive Substance (NPS), has been observed in high concentrations and mixed with cocaine, which may represent a new drug combination (i.e., speedball (cocaine and heroin), twisters (crack and methamphetamine), el diablo (cocaine, heroin, and marijuana)).

Statement of Content/Methods: Three death cases were investigated by the Jefferson County Coroner and Medical Examiner's Office (JCCMEO) between February and May of 2017; each involving n-ethyl pentylone. N-ethyl pentylone (i.e., bk-EDBP, ephylone, Mercedes, Mitsubishi, Lacoste) is classified as a psychostimulant related to cathinone. Stimulants modulate neurotransmitters (i.e.; serotonin, dopamine, and norepinephrine) through increased release or reuptake inhibition. Being structurally related to cathinone, n-ethyl pentylone is proposed to modulate multiple neurotransmitters with overdoses resulting from serotonin syndrome. Effects related to n-ethyl pentylone use range from euphoria, increased alertness, and talkativeness to agitation, tachycardia, hyperthermia, rhabdomyolysis, hypoglycemia, renal failure, and cardiac arrest. Users describe routes of administration as insufflation, intravenous, and oral with the onset of effects occurring within 30 minutes and lasting three to five hours. Due to its current legal status, n-ethyl pentylone can be purchased via the internet in either powder or pill form and has been observed as a component of Neuregulin 1 (NRG-1) and as "Molly."

The first n-ethyl pentylone case encountered by the JCCMEO was a 34-year-old White male who had been observed using "Molly." According to the witness, the decedent became erratic and paranoid. The decedent was later located underneath a car in an auto garage with the scene in disarray -— overturned items, including chairs and stepladders, and papers scattered around the room. The second occurrence was a 34-year-old Black male. This decedent was at a party when he entered into cardiac arrest and was transported to the emergency room. The decedent transpired a few hours later. The third n-ethyl pentylone case was a 25-year-old Black male found lying in the street suffering multiple gunshot wounds. According to neighbors, the decedent was in a feud with the suspect. In all three cases, postmortem specimens (blood, urine, vitreous, bile, liver, and brain) were collected and submitted to the toxicology laboratory. Analyses performed included: volatiles testing, immunoassay screening, and Gas Chromatography/Mass Spectrometry (GC/MS) confirmation/quantification.

Summary of Results: Postmortem toxicology results for the first n-ethyl pentylone encounter were trace levels (<0.01mg/L) of cocaine and cocaethylene and n-ethyl pentylone at 0.953mg/L. Cause Of Death (COD) and Manner Of Death (MOD) were reported as "Acute n-Ethyl Pentylone Toxicity" and "Accident," respectively. The toxicology results for the second occurrence were cocaine at 0.033mg/L, fentanyl at 0.003mg/L, n-ethyl pentylone at 0.121mg/L, methamphetamine at 0.938mg/L, and amphetamine at 0.086mg/L. COD and MOD for case 2 were "Multiple Drug Toxicity from Methamphetamine, Cocaine, Fentanyl, and n-Ethyl Pentylone" and "Accident," respectively. The third case was the result of multiple gunshot wounds (COD) and determined to be a homicide (MOD) but resulted in toxicological findings, including trace levels of hydrocodone, alprazolam at 0.030ng/mL, and n-ethyl pentylone of 0.045mg/L.

Conclusion: Presented here are the first three occurrences of deaths related to n-ethyl pentylone by the JCCMEO. With these deaths occurring in such a short time period, it is shown that the prevalence of n-ethyl pentylone is increasing in the Birmingham area. Furthermore, a potentially deadly combination of n-ethyl pentylone and cocaine has been observed in two of the cases, with both psychological (paranoid and agitated) and physiological (cardiac arrest) effects witnessed. In short, n-ethyl pentylone is a dangerous NPS that should be included in toxicological analyses.

N-Ethyl Pentylone, Novel Psychoactive Substances, Molly

K4 Carfentanil-Induced Fatalities: A Case Series

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The goal of this presentation is to investigate several fatalities associated with carfentanil and to provide a template for medical examiner offices to monitor "designer" opioid deaths through standardized toxicological screening.

This presentation will impact the forensic science community by providing novel carfentanil toxicity levels, considering that the human biological effects of the illicit opioid are unknown.[1]

Carfentanil is a μ-opioid receptor agonist that induces respiratory compromise and central nervous system depression. Its potency is 10,000 times greater than morphine.[2] Used as an elephant tranquilizer, the schedule II drug has concealed itself within the street drug market in North America. Fatalities continue to climb, considering there has been only one published instance of human exposure to an illicitly manufactured version of carfentanil with a successful medical outcome.[3]

This case series will investigate 17 fatal intoxications involving carfentanil, considering the postmortem findings, autopsy results, and toxicological screenings.

A retrospective review of 2,807 deaths was conducted through the Oakland County Medical Examiner's Office database to investigate all potential deaths of carfentanil use. Every public carfentanil-related death in Oakland County, MI, over a six-month period is included in this study. Postmortem specimens for toxicology measurement were extracted from the matrix source of the femoral artery, except for instances of heart blood when no femoral blood was present. Each decedent received a volatile screen through a fentanyl enzyme-linked immune absorbance assay test kit. With a positive confirmation of a fentanyl metabolite, an expanded postmortem, forensic, quantitative blood test was ordered through the National Medical Services Laboratories (NMS Labs). Carfentanil spectra was matched through a spectral library and measured through Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). A standardized case review form was used to characterize each carfentanil-positive death. All available medical and legal history was reviewed to interpret the death.

Sixty-one percent of the decedents were below the age of 35 years. Other than two females, every decedent was male. The cause of death deemed by the medical examiner was drug abuse (n=0), drug intoxication (n=6), and drug overdose (n=1). The manner of death was undeterminable in 16 of the cases, with one exception of suicide by overdose. Only five of the decedents had pre-existing medical conditions that could be speculated as contributory to cause of death. The rest of the decedents were otherwise healthy individuals with no anatomical evidence of trauma or pre-existing disease. Eight of the decedents underwent endotracheal intubation and six were administered naloxone. Five cases were found with a syringe in hand or within arm's reach. Of 11 chronic substance users, seven either actively abused heroin or used within the past six months per family members and friends. Four presented with biventricular hypertrophy and three possessed left ventricle myocardium hypertrophy. The mean combined lung weight was 1,536 grams. Every case possessed moderate to severe pulmonary edema, with remarkable pulmonary congestion. As reported by NMS Labs, every case presented with a unique panel of drugs with a mean carfentanil concentration of 0.384ng/mL. It was previously suggested that 20 micrograms of carfentanil could induce death, although the concentrations reported in the bloodstream at time of death were as low as 10ng/mL.[4] Five cases were found to have 6-monoacetylmorphine, with a mean concertation of 17.66ng/mL. U-4700 was found in conjunction with carfentanil in three instances.

There has been only one other study which provided results from a comprehensive and sensitive screening method, through Ultra High-Performance Liquid Chromatography (UHPLC) -Ion Trap-MSn, to identify carfentanil.[5] That study only provided detection results, without quantification values. The methods presented in the current study could be employed as a template for other medical examiner offices, as LC/MS/MS can reduce toxicological discrepancies between cases and detect low concentrations.

Hopefully, the cases presented in this study will provide a foundation for further studies examining carfentanil's toxicological characteristics. Future studies are warranted to investigate the epidemiological trends of this perilous opioid.

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Carfentanil, Postmortem Concentration, Overdoses

K5 An Assessment of the Incorporation of Amphetamine and Diazepam Into Human Head Hair for the Preparation of Hair Reference Material

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After attending this presentation, attendees will better understand the process of preparing Hair Reference Material (HRM) with incorporated xenobiotic substances.

This presentation will impact the forensic science community by contributing to a body of research targeted at better understanding the interactions between drugs of abuse with different physiochemical properties and hair that would be considered during routine analysis in forensic laboratories.

HRM is essential for the development and validation of methodologies used in forensic hair analysis. At present, HRM containing selected xenobiotic substances is only available on a limited basis. In addition, most xenobiotic substances relevant to forensic casework are not available as incorporated drug standards in human head hair. Additionally, little is known about the incorporation process that occurs when human head hair is soaked in a xenobiotic-containing buffer solution. This body of work demonstrates the intentional incorporation of Amphetamine (AMP) and Diazepam (DZP) into human head hair for the development of "in-house" HRM by exploring the relationship between time and the concentrations of drug detected in the incorporation buffer solution, daily hair extracts, and solutions used to wash daily hair aliquots. The hypothesis was that, over time, the concentration of xenobiotic detected will decrease in the buffer solution, increase in the hair extracts, and remain relatively constant in the wash solutions.

Purchased human head hair was soaked in 1X Phosphate-Buffered Saline (PBS) spiked with either AMP or DZP at 800pg/mL for five days (120h). Each day, aliquots of hair and spiked PBS solution were taken to monitor incorporation. The hair aliquots were washed using both organic (2-propanol) and aqueous (1X PBS) solvents, then pulverized using a Retsch® MM200 ball mill. Subsequently, the pulverized hair was incubated in a mixture of organic and aqueous solvents (methanol:cetonitrile: 2mM ammonium formate in water; 1:1:2) for 18h to extract incorporated drug from the matrix. After incubation, the samples were centrifuged to separate the hair particulates from the solvent mixture containing recovered drug. The resulting solution was subjected to online Solid-Phase Extraction (SPE) cleanup and Liquid Chromatography/Triple Quadrupole/Tandem Mass Spectrometry (LC/QqQ-MS/MS) analysis. Mass Spectrometry (MS) analysis was performed on the spiked PBS solutions, extracted samples, and wash solutions. A 1µL injection of each sample was introduced to an Agilent® 1290 Infinity Flexible Cube to perform online SPE. A reversed-phase LC column (Agilent® ZORBAX® Rapid Resolution High-Definition Eclipse Plus C18, 2.1 X 50mm, 1.8µm) was used as the analytical column on an Agilent® 1290 Infinity® HPLC system. A gradient elution was used over 8min using 5mM ammonium formate in water with 0.1% formic acid (A) and methanol with 0.1% formic acid (B). Analysis was performed with positive mode Electrospray Ionization (ESI) on an Agilent® 6460 QqQ-MS instrument.

Quantitative results demonstrated the successful incorporation of AMP and DZP into blank human head hair. Contrary to the hypothesis, the amount of drug extracted from the hair aliquots was not significantly different between the 24h and 120h time points for either drug. Final incorporated drug levels were approximately five-fold higher for AMP than DZP. The organic wash did not remove a significant quantity of AMP, but did remove DZP from the surface of the hair. The three aqueous washes each removed AMP from the hair surface, with decreasing concentration in each wash. In contrast, the organic wash and the first of the aqueous washes removed DZP, while subsequent aqueous washes did not remove any additional drug. These results suggest that the maximum transfer of drug from the incorporation buffer into hair occurs within the first 24h of incubation for both drugs. In addition, AMP may be more prone to contaminating the surface of the hair or it may be more loosely bound to the hair matrix than DZP. The differences between incorporation, decontamination, and extraction of these two drugs may be attributed to differences in their physiochemical properties.

Hair Analysis, Hair Reference Material, Incorporation of Xenobiotics

K6 A Mass Spectrometric Approach to the Analysis of Covalent Modifications of Blood Proteins by Drugs of Abuse

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After attending this presentation, attendees will better understand *in vitro* formation of covalent protein modifications formed by reactive drug metabolites, as well as the Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) analytical approach required for detection of these adducts.

This presentation will impact the forensic science community by demonstrating that covalent protein adducts formed *in vitro* provide the necessary framework for an *in vivo* detection method under development for the retrospective detection of drugs of abuse in human blood.

Hemoglobin (Hb) and serum albumin (SA), two prevalent proteins in human blood,- contain unbound cysteine thiol moieties, creating a nucleophilic site with the potential for covalent modification by reactive chemical species. These covalent modifications, called "adducts," are stable entities that accumulate during acute and chronic exposure and remain covalently bound for the life-span of the protein. Despite their current use as exposure markers for a variety of compounds, the use of adducts in assessing exposure to drugs of abuse has not yet been explored. The goal of this work is to examine the *in vitro* adduct-forming capability of selected drugs of abuse with Hb and SA to provide additional proof of principle for the development of a real-world detection and monitoring analysis method. Use of protein adducts as biomarkers of drug exposure will allow for an increased window of detection, from several days to several months, as compared to current blood analysis methods. The drugs examined in this study cover a wide range of abused drugs, including cocaine, methamphetamine, and Δ^9-THC, and have all been shown in previous work in the laboratory to form adducts with glutathione and/or other thiol-containing peptides.

For this research, a new assay procedure was created to facilitate recovery of modified protein by combining published methods with existing methodology used in the lab. The new assay utilized a dialysis membrane to maintain separation of proteins of interest from the microsomal components, while allowing for small molecules (i.e., stable and reactive metabolites) to pass through, resulting in a decrease in the number of steps required to extract the modified protein of interest. For the metabolism/adduction assay, each drug was added to a plastic microfuge tube with residual solvent removed via vacuum centrifuge. Human liver microsomes were added to the tube and combined with Nicotinamide Adenine Dinucleotide Phosphate (NADPH) in the presence of a regeneration system containing glucose-6-phosphate and glucose-6 phosphate dehydrogenase, in sodium phosphate buffer (pH 7.4). The protein of interest was then added; the tube was incubated at 37°C for 18h, then centrifuged. An aliquot of supernatant was removed and added to a clean LC/MS vial for analysis. Instrumental analysis of modified protein was performed using positive Electrospray Ionization (ESI) on an Agilent® 1290 Infinity® Ultra High-Performance Liquid Chromatography (UHPLC) coupled to an Agilent® 6530 MS and chromatographic separation utilized an Agilent® ZORBAX® Rapid Resolution HD Eclipse® Plus C8 column. Data were collected using full MS scan mode, to allow for necessary analysis of all protein components. The mobile phases used were as follows: (1) water with 0.1% trifluoroacetic acid; and, (2) 95% acetonitrile, 4.9% water, 0.1% trifluoroacetic acid. The total run time was 16 minutes with a 2-minute post-run for column re-equilibration. Initial analysis of MS data obtained was performed using Agilent's® MassHunter™ Qualitative Analysis software, followed by MassHunter™ BioConfirm software for protein deconvolution and proteomic analysis of adducts formed.

The use of protein adducts for retrospective drug detection represents a novel and useful advance in drug testing and analysis. The characterization of covalent adducts formed *in vitro* shown in this research provides the necessary framework for future examination of a more complete set of abused drugs. The confirmation and subsequent analysis of these covalent protein adducts reinforces the need for the development of a real-world applicable method to screen for drug exposure utilizing a longer window of detection than is currently available for most drugs and matrices.

Protein Adducts, Drugs of Abuse, LC/MS

K7 Hydrogen Sulfide (H_2S) Poisoning in the Workplace: Toxicological Investigations in a Fatal and Non-Fatal Accident

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After attending this presentation, attendees will understand that in cases of occupational asphyxiation, it is important to both conduct a complete postmortem toxicological analysis and to perform accurate scene investigations supported by environmental-toxicological monitoring.

This presentation will impact the forensic science community by highlighting the usefulness of the measurement of thiosulfate that represents an important step in a toxicological investigation of H_2S poisoning because it can supply information regarding the time of death and toxicokinetics.

H_2S is a toxic gas involved in deaths in the workplace. Few cases have been reported in the literature and the accident occurs predominantly in the sour gas industry and in other industrial settings.

The reported case regards fatal and non-fatal intoxications due to H_2S asphyxiation involving six seamen who were working on a ferryboat.

Accident scene reconstruction revealed that three workers had to remove the waste fluid from a bilge of the ferryboat. The first victim unscrewed bolts of the bilge manhole and in a few minutes fell unconscious. Two other seamen who saw what happened asked for help and approached the subject, but they too fell unconscious. Three other workers arrived to move the three unconscious seamen and only one had a protective mask. The first victim died in the workplace, the second one died in the ambulance, while the third one died in the emergency room. Of the remaining three workers, one had a severe pulmonary edema that required intensive treatment, the second had pulmonary injuries also involving the heart, and the third, who wore the mask, had lung injuries.

Toxicological environmental analyses were performed two hours after the accident on air samples from the bilge, the area in front of the bilge manhole, and on the waste fluid collected from the bottom of the bilge. The analyses revealed high levels of H_2S in all specimens.

Autopsies were performed after 48 hours and the findings were the same for all the victims, showing edema of the lungs and multi-organ congestion.

Toxicological analyses were performed both on venous blood taken from the living subjects on arrival at the emergency room and on femoral blood and urine taken during autopsy to evaluate the presence of volatile hydrocarbons, carbon monoxide, hydrogen cyanide, and H_2S. Results from the surviving workers were negative and, in particular, H_2S concentrations were below the quantification limit, being already biotransformed and eliminated in the urine, either in the form of unmodified sulfide or as thiosulfate. In the deceased workers, the toxicological investigation was positive for high levels of H_2S; thus, thiosulfate research was performed to distinguish between the H_2S concentrations in blood secondary to lethal poisoning and those produced by a putrefactive phenomena. Thiosulfate evaluation revealed significant levels in femoral blood (45.7µg/ml) and even more in the urine (510.1µg/ml) belonging to the first decedent. Lower levels were observed in samples from the second decedent (blood: 35.5µg/ml; urine: 10.4µg/ml). Even lower levels were determined in the blood of the third decedent (21.5µg/ml) and no measurable concentrations were in the urine.

These findings demonstrated that the seamen died from asphyxia due to H_2S poisoning. Moreover, the toxicological results allowed for the evaluation of several different survival times. In fact, the first seamen survived for a longer time because metabolism of much of the inhaled sulfides had occurred and a high amount of metabolite (thiosulfate) had eliminated in the urine. The second worker survived briefly and was able to metabolize a smaller amount of sulfides and to eliminate a small amount of thiosulfate in the urine. Finally, the third survived for a very short time because the urine thiosulfate was negative.

This presentation also highlights the importance of safety devices used as well as compliance to the relative Italian legislation.

Hydrogen Sulfide, Toxicological Analysis, Occupational Accident

K8 The Development of a Rapid Multi-Target Screening Method for Emergency Toxicology by Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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After attending this presentation, attendees will understand a rapid simultaneous screening method using GC/MS and LC/MS/MS for the determination of multiple toxicants in urine samples collected from intoxicated patients in emergency rooms.

This presentation will impact the forensic science community by informing attendees of the rapid method for simultaneous screening of multiple toxicants by GC/MS and LC/MS/MS that were established to determine targeted and unknown toxicants in urine.

From February 2015 to March 2017, 265 urine samples were collected from the Chungnam University Hospital emergency room. Urine samples were cleaned by using Waters® Ostro™ (pass-through type) and examined by GC/MS and LC/MS/MS. After analysis by GC/MS, the library search for unknowns was conducted by in-house mass spectral databases with the Automated Mass spectral Deconvolution and Identification System (AMDIS). In addition, Chemstation® software was mobilized to identify toxicants. For LC/MS/MS analysis, the 3200 QTRAP® LC/MS/MS and Cliquid® software was used for a simultaneous multi-targeted screening.

A rapid multi-target screening method by GC/MS and L/-MS/MS was developed to determine toxic substances in urine. By using Ostro™ extraction and an in-house database, it was possible to screen urines for toxic substances within three hours. With this method, 265 urine samples were examined and it was noted that zolpidem, acetaminophen, and citalopram were detected in 49, 29, and 16 cases, respectively, and those were the most frequently encountered drugs in emergency room patients. The targeted and unknown toxicants were well searched by in-house and commercial mass spectral databases in all specimens studied. AMDIS & Chemstation® software were used for GC/MS analysis and Cliquid® 2.0 software was used for LC/MS/MS analysis.

The rapid multi-target screening methods by GC/MS and LC/MS/MS developed in this study proved to be applicable to the actual hospital poisoning samples. This method can be efficiently used to detect toxic substances within three hours in emergency cases.

Multiple Toxicants, Targeted and Unknown Toxicants, Emergency Toxicology

K9 A Concentration of Biomarkers in Vitreous Humor for the Estimation of Postmortem Interval (PMI) in South Korea

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After attending this presentation, attendees will understand which biochemical markers in vitreous humor can be measured by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) for the PMI and the correlation between the levels of biochemical markers and PMI in South Korea.

This presentation will impact the forensic science community by examining the correlation between the concentrations of Hypoxanthine (Hx), potassium, and time after death. This will be useful information for the estimation of PMI.

To choose the possible biomarkers for PMI, concentrations of Hx and lactic acid in vitreous humor were measured by LC/MS/MS. Hx is the terminal stage of purine catabolism in man and is known to be highly correlated to PMI. Also, in mammalian cells, lactate is formed from pyruvate in carbohydrate metabolism under anaerobic conditions. Lactic acid is chiral, consisting of two optical isomers. Because L-lactic acid is known to be correlated with PMI, it was targeted in this study.

A vitreous humor was collected from a cadaver with a known time of death between 18 hours and 103 hours at the National Forensic Service (NFS) in Korea. Twenty-one samples were comprised of 16 males and 5 females with an age range of 27-84 years. Vitreous humors were extracted by a solid-phase extraction with Oasis® MAX cartridges. Agilent® 1260 infinity HPLC system and Sciex® 3200 QTRAP® MS were used for the quantification of Hx, uric acid, and lactic acid in vitreous humor. Chromatographic separation was performed by using 0.1% formic acid in water and methanol as the mobile phase. The Multiple Reaction Monitoring (MRM) of ion transitions monitored was m/z 137.0>110.0, 119.0 for Hx and 5-(p-methylphenyl)-5-phenylhidantoin 267.2>163.3 as the Internal Standard (IS). Lactic acid was separated into L-lactic acid and D-lactic acid by derivatization, and (+)-O,O`-diacetyl-L-tartaric anhydride (≥97%) (DATAN) were used as derivatization reagents. The MRM of ion transitions monitored was m/z 308.0>89.0 for L-, D-lactic acid and L-lactate-3,3,3,-d3 308.1>92.1 as IS for lactic acid.

Analysis of 21 vitreous humor samples revealed that the concentration of Hx ranged from 327μM to 1,780μM and well correlated with the PMI. The Hx concentrations increased gradually until 96 hours, indicating the concentration of Hx and time after death are well matched. The differences in HX concentration between gender and age were not noted. The concentration of potassium was also well related with PMI, while creatinine, Blood Urea Nitrogen (BUN), sodium (Na), and chlorine (Cl) were not correlated with PMI. Lactic acid was separated into L-lactic acid and D-lactic acid through its derivatives.

The correlation between the concentrations of Hx, potassium, and time after death will be very useful information for the estimation of PMI.

Postmortem Interval, Hypoxanthine, Potassium

K10 Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) Extraction of Novel Psychoactive Substances (NPS) From Biological Matrices

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The goal of this presentation is to describe the findings of using QuEChERS as a quicker and less expensive alternative for the extraction of NPS from biological matrices. This presentation will cover a broad range of drug classes and the trends observed. An optimized extraction method that encompasses as many NPS as possible will also be presented.

This presentation will impact the forensic science community by presenting an alternative to currently used extraction techniques for NPS that includes added benefits, such as decreased costs and time spent on sample preparation.

This presentation is intended to demonstrate the potential of QuEChERS ("catchers") as an extraction technique for various NPS in biological fluids (urine and whole blood). Although extraction techniques for common drugs of abuse are well studied, developing extraction methods specifically targeting NPS is needed due to the high prevalence of NPS in forensic casework. A validated screening/confirmatory triggered Multiple Reaction Monitoring (tMRM) method for 826 NPS by Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS) recently developed in the lab is being used to analyze all extracts for this study. The method allows for the screening of a wide variety of NPS drug classes and metabolites, with a focus on synthetic stimulants and cannabinoids due to their current importance in forensic toxicology casework.

Most biological fluids require an extraction step before analysis to avoid unwanted matrix effects and to protect instrumentation. Some commonly used extraction/purification techniques are Solid Phase Extraction (SPE), Liquid-Liquid Extraction (LLE), and dilute/crash and shoot. These techniques can be expensive, time consuming, and may not eliminate all matrix effects. Extraction methods ideally should be inexpensive and relatively fast to allow for high throughput in forensic toxicology labs.

QuEChERS has the potential to be a desirable alternative to these techniques because it can decrease overall costs, matrix effects, and time. QuEChERS was originally developed to simplify and speed up sample prep to extract pesticides from fruits and vegetables. QuEChERS uses a dispersive SPE (d-SPE) technique that increases the sample's contact with the sorbents. This increased contact allows for a more effective extraction than classic SPE. It is specifically designed for highly aqueous matrices and, therefore, can be a very useful technique for forensic toxicology work. While QuEChERS has been previously used as a technique for extracting common drugs of abuse from urine and blood, to date it has not been applied to extraction of NPS on this scale.

In this study, a modified Bond Elute kit was utilized. Blank human urine spiked with 29 different NPS at three different concentrations (5ng/mL, 20ng/mL, and 80ng/mL) was used for this work. The mixture included NPS from different drug classes, including synthetic cannabinoids, synthetic cathinones, tryptamines, and phenethylamines. The range of concentrations was selected to test the utility of the technique for authentic samples, which may contain very low concentrations of these compounds. The extraction process consisted of two major steps: a drying step with salts, followed by d-SPE. First, 3mL of the spiked urine was combined with 3mL acetonitrile, followed by the addition of salts (magnesium sulfate and sodium acetate) to dry the sample, which was then centrifuged at 4,400rpm for 5min. Then, 1mL of the acetonitrile layer was used for d-SPE. The acetonitrile was added to a centrifuge tube containing primary secondary amines, magnesium sulfate, and CI8 and centrifuged at 4,400rpm for 5min. A 100µL aliquot of the resulting supernatant was diluted with 100µL of internal standard mix and 300µL of High-Performance Liquid Chromatography (HPLC) water for analysis by LC/QqQ/MS. The total time for extraction was 15min, which is an improvement over most other extraction methods. An Agilent® 1290 Infinity® HPLC system and Agilent® 6460 QqQ/MS with Jet Stream Technology ESI was used with an Agilent® ZORBAX® Rapid Resolution HD Eclipse® Plus C18 column for LC/MS/MS using the tMRM method.

Results demonstrate that QuEChERS is a promising technique for the extraction of tryptamines, phenethylamines, and synthetic cannabinoids, all of which exhibited recoveries >85% at all three concentration levels. This work is being continued with whole blood and additional NPS to further assess the technique's potential. QuEChERS is capable of extracting NPS from multiple drug classes in a single urine specimen and represents an appealing alternative to other extraction methods due to fewer transfer steps, quick extraction time, and decreased cost.

QuEChERS, Novel Psychoactive Substances, LC/MS

K11 A Validated Method for the Quantitative Determination of Zolpidem, Zopiclone, and Zaleplon (ZZZ Drugs) in Blood, Stomach Contents, and Liver by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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After attending this presentation, attendees will better understand a validated method for the quantitation of ZZZ drugs in blood, stomach contents, and liver by basic Liquid-Liquid Extraction (LLE) and LC/MS/MS.

This presentation will impact the forensic science community by describing a method validation to rapidly and simultaneously confirm all three ZZZ drugs with matching deuterated internal standards (zolpidem-D6, zopiclone-D4, and zaleplon-D4).

Zolpidem, zopiclone, and zaleplon are sedative hypnotics.[1] Due to their rapid onset of action and short half-lives, ZZZ drugs have become the standard alternative to short-acting benzodiazepines for the treatment of onset and maintenance forms of insomnia.[2] ZZZ drugs are GABA agonists and their poly-use with benzodiazepines, ethanol, or other Central Nervous System (CNS) depressants can increase impairment and lead to toxicity or death.[3] If taken in compliance, the drugs should exist at or below therapeutic concentrations with little to no residual effects upon waking. ZZZ drugs are commonly detected both independently and in conjunction with benzodiazepines and ethanol in Driving Under the Influence of Drugs (DUID) and postmortem cases.[4] Current methods for the detection of ZZZ drugs in blood are generally performed with Ultra High-Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS) either in tandem with benzodiazepines or are not quantitative for all three analytes.[1,3,5] The goal of this work was to validate a method for the quantification of zolpidem, zopiclone, and zaleplon by LC/MS/MS.

Both acidic and basic LLE methods were investigated during method development. The basic extraction yielded higher area counts, more uniform peak symmetry, and easier isolation of the organic layer. The basic extraction method utilized saturated sodium borate (pH 12), ethyl acetate, and matching deuterated internal standards. An Agilent® 1290 Infinity® II Stack and 6460 Triple quadrupole/Mass Spectrometry (QqQ/MS) system was employed in positive electrospray ionization mode with Multiple Reaction Monitoring (MRM) transitions selected by the Agilent® Optimizer program. Separation was achieved on an Agilent® InfinityLab Poroshell 120 EC-C18 column (3.0mm x 100mm, 2.7µm) with a 0.6mL/min flow rate of 0.1% formic acid in H_2O (A) and 0.1% formic acid in CH_3CN (B). The gradient was initialized at 20% B for 0.8min, increased to 45% B over 0.8min, and 95% B over 1min. An isocratic hold was placed at 95% B for 1.5min, followed by a decrease to 20% B over 0.3min, for a total run time of 4.7min. Method validation was conducted according to the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines.

Seven non-zero calibrators were used to establish a 10ng/mL-1,000ng/mL working range with a 1/x weighting factor. Standard residual plots indicated that zopiclone was best fit with a linear model while zolpidem and zaleplon required a quadratic model. Studies assessing the limit of detection are currently underway and the Limit Of Quantitation (LOQ) was set at the lowest non-zero calibrator (10ng/mL) as ZZZ drugs tend to be found, in blood, at concentrations exceeding the lowest calibrator.[2,6] Carryover following the 1,000ng/mL calibrator was determined to be 0.35%, 0.23%, and 1.28% of the LOQ for zolpidem, zopiclone, and zaleplon, respectively. Three concentration levels (25ng/mL, 400ng/mL, 750 ng/mL) in triplicate were used to determine the bias and precision. Bias and precision were calculated at ≤15% for all three analytes, concentrations, and matrices, with the exception of zopiclone in stomach contents at 17.3% CV. Ion suppression was assessed by a post-extraction spike of mobile phase and negative blood, stomach content, liver, and urine matrices at low and high Quality Controls (QCs). Although ion suppression was present at values exceeding SWGTOX guidelines, it had no impact on accuracy or precision of quantitation. No significant interferences were present in mobile phase samples spiked with common drugs of interest (fentanyl, opiates, cocaine, diphenhydramine, trazodone, buspirone, PCP, 3-MeO-PCP, dextromethorphan, ketamine, duloxetine, venlafaxine, tramadol/ nortramadol, amitriptyline/ nortriptyline, clozapine, doxepin/nordoxepin, fluoxetine/norfluoxetine, olanzapine, quetiapine/norquetiapine). The selected MRM transitions were not triggered by endogenous compounds in negative human blood, stomach contents, liver, or urine matrices. Bias and precision of dilution integrity fell ≤20% and was monitored by preparing a 1,500ng/mL ZZZ calibrator and diluting in triplicate (x25, x10, x4) in negative blood. The preceding validation method meets the requirements of SWGTOX guidelines.

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K12 Performing Retrograde Extrapolation of Blood Alcohol in Driving Under the Influence (DUI) Trials

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After attending this presentation, attendees will understand the need to consider relevant factors before performing retrograde extrapolation in DUI trials.

This presentation will impact the forensic science community by increasing awareness that retrograde extrapolation must be performed with caution to ensure that a reliable calculation is obtained. Data from a Las Vegas Metropolitan Police Department (LVMPD) drinking study will be used to illustrate this point.

In the Illinois case of *People v. Floyd*, the defendant was convicted of aggravated DUI and resisting arrest.[1] The defendant's DUI conviction was later reversed and remanded for a new trial by the appellate court. The decision to reverse the conviction was due to an assumption made by the State's expert witness while performing retrograde extrapolation that the defendant was in the post-absorptive phase without considering all relevant factors. Although the ruling recognizes that it does not "[create] a blueprint or a bright-line rule for the admissibility of retrograde extrapolation evidence," it emphasizes the fact that retrograde extrapolation must be carefully conducted.[1]

To demonstrate this point, a drinking study involving 12 subjects (6 males and 6 females), between the ages of 23-35 years, was conducted. Nine of 12 subjects consumed food within 2.5 hours of the start of drinking. Two subjects consumed food more than four hours prior to the start of drinking, and the time of the last meal was not provided for one subject. Hard liquor was consumed over 1.5-3 hours. Three blood draws were taken from each subject approximately 1, 2, and 3 hours following the end of drinking.

Each blood sample was collected in a 10mL gray-stoppered glass blood tube and was stored at 2ºC-8ºC from the time of collection to the time of analysis. The blood samples were analyzed using a dual column headspace gas chromatograph with two flame ionization detectors. The method was validated following the Scientific Working Group for Forensic Toxicology (SWGTOX) standard practices.

A plot of Blood Alcohol Concentration (BAC) as a function of time was generated for each subject, and the elimination rate was determined through linear regression analysis. Of the 12 subjects, 2 appeared not to be in the post-absorptive phase at the time of the first blood draw. Elimination rates for these 2 subjects were determined through linear regression analysis using only second and third blood draw data. Mean elimination rates of 0.020g/100mL/h (range: 0.015g/100mL/h-0.024g/100mL/h) and 0.018g/100mL/h (range: 0.007g/100mL/h-0.027g/100mL/h) were obtained for male and female subjects, respectively.

For retrograde extrapolation, the elimination rate and rate range considered were 0.015g/100mL/h and 0.010g/100mL/h-0.035g/100mL/h.[2] Assuming that each subject was in the post-absorptive phase at the time of the first blood draw, extrapolating to that time using the second blood draw data overestimated the BAC of one subject. This was the case whether the 0.015g/100mL/h or 0.010g/100mL/h-0.035g/100mL/h elimination rate/rate range was used. Furthermore, retrograde extrapolation to the time of the first blood draw based on the third blood draw data, using the 0.015g/100mL/h elimination rate, marginally overestimated BACs of 2 subjects; however, similar retrograde extrapolation using the 0.010g/100mL/h-0.035g/100mL/h elimination rate range did not overestimate any BAC.

Based on the LVMPD drinking study data and the *People v. Floyd* appellate ruling, it may not always be appropriate to assume that a subject is in the post-absorptive phase at the time of the incident. The BAC may be overestimated if relevant factors, such as the drinking scenario, food consumption, and circumstances surrounding the incident, are not considered. Retrograde extrapolation is an effective tool in determining the BAC at an earlier time when it is carefully conducted.

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Forensic Toxicology, Retrograde Extrapolation, DUI

K13 The Identification of Five Kratom Alkaloids Using High Resolution Mass Spectrometry

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After attending this presentation, attendees will be able to identify and separate kratom alkaloids using Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS). The influence of mobile phase additives and adduct formation will be discussed and common fragmentation pathways of corynanthe-type alkaloids will be explored.

This presentation will impact the forensic science community by highlighting the importance of mobile phase selection, optimization of ionization conditions, and structural identification of fragment ions using high-resolution MS.

Mitragynine (MG) (9-methoxycorynantheidine, kratom) and 7-hydroxymitragynine (MG-OH) are naturally occurring corynanthe-type indole alkaloids present in the leaves of *Mitragyna speciosa*. This flowering plant of the *Rubiaceae* genus contains more than 20 alkaloids, of which mitragynine is the principal pharmacologically active component, with 7-hydroxymitragynine being a minor psychoactive constituent. Mitragynine and 7-hydroxymitragynine are μ-opioid agonists. Kratom also contains two diastereoisomers of mitragynine (speciociliatine and speciogynine) and paynantheine. Although these three compounds are not known to be psychoactive, their presence in biological specimens may indicate kratom use. Although not yet federally regulated, kratom's dual stimulant and opiate-like effects are somewhat unique, making it an ideal candidate for misuse among recreational drug users.

Separation and identification of MG, MG-OH, Speciociliatine (SC), Speciogynine (SG), and Paynantheine (PY) in biological samples presents a significant analytical challenge. LC/qTOF/MS is a high-resolution MS technique that offers high sensitivity and significant benefits in terms of mass accuracy and structural identification. Mobile phase composition and optimization of the ionization conditions is essential in order to achieve high sensitivity and adequate chromatographic separation. Tandem Mass Spectrometry (MS/MS) spectra can provide valuable structural information. Characterization of fragmentation pathways and identification of ions is important for new assay development.

During the development of an analytical method for MG, MG-OH, PY, SC, and SG in urine, a total of three mobile phase additives were evaluated in deionized water/acetonitrile: 0.1% formic acid; 10mM ammonium formate, and 5mM ammonium acetate. Chromatographic resolution, ionization efficiency, and the formation of adducts were investigated. Fragmentation pathways for MG, MG-OH, PY, SC, and SG were elucidated. MS/MS spectra were used to identify fragments and make mass assignments. Ultimately, this process plays an important role in the selection of highly specific precursor ion transitions. A total of three transitions were selected for each of the compounds.

The most abundant product ions for all compounds were associated with C-ring cleavage and the loss of the substituted piperidine (D-ring) between C2 and C5. The abundance and specificity ultimately led to this being selected for quantitation purposes for MG (399→174), MG-OH (415→190), SC (399→174), SG (399→174), and PY (397→174). Variations of C-ring cleavage predominated for all other major product ions, as well as formation of intact substituted piperidine ions.

Chromatographic separation and mass spectral acquisition are particularly important analytical variables due to the potentially large number of structurally similar alkaloids and diastereoisomers found in *M. speciosa*. LC/qTOF/MS and other high-resolution MS techniques are particularly useful for complex analytes such as these.

Kratom, Fragmentation, LC/qTOF/MS

K14 Conformational Considerations of Ethylenediamine Opioids AH-7921 and U-47700

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After attending this presentation, attendees will be able to recognize potentially confounding spectra attributable to conformational preference or slow conformation interchange of the amide bond in the AH- and U-series opioids.

This presentation will impact the forensic science community by informing attendees regarding spectroscopic issues that arise from conformational aspects seen in the related AH-7921 and U-7700 series opioids.

The published Structure Activity Relationships (SAR) for the AH-7921 series opioids demonstrate a preference for N-monosubstituted benzamides with hydrogen attached to the amide nitrogen. These types of analogs should favor a *trans* amide bond preference.[1] Conversely, U-47700 analog (SAR) indicates a preference for a methyl group on the corresponding amide nitrogen, which allows for *cis* and *trans* amide conformations.[2] The hypothesis is that this preference may be due to each series having the opposite amide bond configuration when bound to the mu-opioid receptor. Preliminary molecular modeling studies that explore this hypothesis by looking at preferred conformations for examples for each of the series and the potential for overlap of key functional groups between the AH-series and the U-series compounds will be presented.

The studies presented will highlight issues that an analyst may encounter that may cause confusion due to data that could be misinterpreted as a mixture. For example, peak doubling in the Nuclear Magnetic Resonance (NMR) due to slow interchange between *cis* and *trans* amide conformations may mislead one into thinking the sample is impure. It has long been documented in the literature that unsymmetrically N,N-disubstituted alkyl amides can exhibit both *cis* and *trans* amide bond conformations due to the lower barrier for rotation and diminished steric preference compared to N-monosubstituted alkyl amides.[3]

Analogs within the AH-7921 and U-47700 series will be analyzed by a variety of methods, including NMR, Gas Chromatography/Mass Spectrometry (GC/MS), Infrared (IR), Raman, etc., particularly regarding indications of conformational preference and potential for rotational interchange. It is well known that simple N-monosubstituted amides that are not conformationally restrained prefer the *trans* amide configuration. The AH-7921 analogs fall into this category. The U-47700 analogs are N,N-disubstituted alkyl amides and demonstrate both *cis* and *trans* configurations as evidenced in their NMR spectra, since the rotational interchange of the amide bond is slower than the NMR time scale. The conditions and the degree to which this phenomenon is observed will be described. The binding of the respective *cis* vs *trans* isomers to the mu-opioid receptor is not known, but it is likely one of the isomers would be preferred by the receptor over the other just as any flexible drug molecule binds to its receptor with a preferred conformation. Also, it is known from structure activity relationships for the U-series analogs that small changes in structure can lead to significant changes in receptor selectivity (e.g., phenylacetamides as kappa-receptor agonists vs. benzamides as mu-receptor agonists).[2]

In conclusion, occasionally even simple compounds can present the analyst with potentially confusing data, so awareness of not only stability issues (e.g., UR-144 degradation in the GC), but also conformational influences on chromatography and spectroscopy is important.

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Synthetic Opioids, AH-7921, U-47700

K15 The Transformation of Drug/Metabolite Ratios: An Objective Assessment of Toxicity

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After attending this presentation, attendees will be aware of the use of a simple mathematical transformation of urine test results to afford an objective assessment of toxicity through the ratio of drug to metabolite (e.g., fentanyl to norfentanyl). Comparison of postmortem drug(s) to metabolite ratios from urine to a large database of such ratios from urine drug testing can help provide this objective assessment of toxicity.

This presentation will impact the forensic science community by demonstrating that the mathematical transformation described in this report can provide a tool to help with the assessment of toxicity in postmortem examinations. These data are necessarily from urine in which "normal" population data exists from pain medication testing. This will require a testing paradigm shift in postmortem samples in which often only the parent drug is tested in various bodily fluids.

Forensic assessment of toxicity is often subjective. For example, it is expected intuitively that fentanyl concentrations from postmortem samples are higher than corresponding therapeutic levels, whether in blood or urine. In fact, many forensic laboratories only test for fentanyl and not the metabolite, norfentanyl. Ruan et al. suggested that the absolute value of the ratio of norfentanyl to fentanyl could be related to the probability of "acute fentanyl toxicity."[1] This work reports that a simple logarithmic transformation of the ratio of parent drug concentrations to metabolite concentrations from a large body of therapeutic test results in urine can afford a stable database for comparison with forensic (postmortem) sample results treated the same way. The stable database for comparison is readily available from urine data obtained during pain medication monitoring testing.[2] Similar databases from blood testing are not available. While this approach can work for any number of potentially toxic drugs, the transformed ratios of fentanyl to norfentanyl in urine from several postmortem cases were compared with the "normal" therapeutic distribution of such transformed ratios to confirm this model/hypothesis. Urine fentanyl/norfentanyl data from overdose cases reported by Coopman et al., Peer et al., and Poklis et al. were similarly transformed to compare with the existing pain monitoring population (Figure 1).[3-5] There is some apparent overlap between the populations, but the overdose population is higher than the "normal" therapeutic results as predicted by Cummings et al.[6] Additional fentanyl/norfentanyl urine data from postmortem samples is expected to more clearly delineate that population of results in contrast to the therapeutic distribution. Thus, this simple transformation of the test results can provide an indicator of toxicity. Of course, the total setting of the death must be taken into consideration, but this approach could serve as an additional tool for forensic scientists in their investigations.

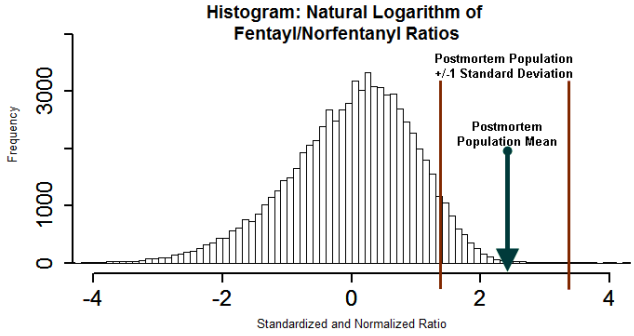

Figure 1.

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Fentanyl, Transformation, Postmortem

K16 The Electroanalytical Identification of 25I-NBOH and 2C-I Via Differential Pulse Voltammetry: A Rapid and Sensitive Screening Method to Avoid Misidentification

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The goal of this presentation is to present a new electrochemical method to identify 25I-NBOH, a new, potent serotonin 5-HT2A receptor agonist usually identified in blotter paper.

This presentation will impact the forensic science community by introducing a new, selective, and sensitive method for the identification of 25I-NBOH, a compound that is usually misidentified by routine Gas Chromatography/Mass Spectrometry (GC/MS) methods.

Recently, a new potent serotonin 5-HT2A receptor agonist was identified in blotter paper seizures in Brazil.[1] This compound, named 25I-NBOH, is a label molecule that undergoes degradation when examined under routine GC/MS conditions, leading to misidentification as it degrades into 2C-I, an amphetamine-type stimulant.[2] The prevalence of this substance on the Novel Psychoactive Substances (NPS) market can be underestimated under GC/MS conditions, the most widely and routinely utilized analytical technique for drug sample analyses, as it can misidentify 25I-NBOH because of its degradation into 2C-I (and corresponding 2C for the other members of the series).[2] Despite many attempts in adjusting GC/MS conditions and even changing the extraction solvent, Coelho Neto et al. stated that degradation could not be avoided.[2] The degradation takes place inside the GC/MS injector and appears to be caused by the high temperature inside the injector with the degradation products reacting with the alcohol used in the extraction procedure.[2] Another recent study described the analytical determination of phenethylamines derivatives; compounds of the NBOMe group via cyclic and differential pulse voltammetry.[3] Noting that 25I-NBOH has only a single modification regarding 25I-NBOMe, a substitution of a metoxy group for a hydroxy group in the position 3 of the secondary aromatic ring, a very sensitive and specific method to identify 25X-NBOH avoiding misidentification as 2C-X of this class of compounds was developed.

The voltammetric behavior of 25I-NBOH and 2C-I were investigated and their electroanalytical characteristics determined. The investigation of the electrochemical behavior by Cyclic Voltammetry (CV) using a carbon Screen-Printed Electrode (SCPE) showed two oxidative waves observed at +0.74 V and +1.09 V for 25I-NBOH and one single oxidative wave at +1.20 V for 2C-I. The first oxidative peak is a result of the electrochemical oxidation of the secondary amine present in the NBOH compound and the second oxidative wave is due to the halogen oxidation to a hydroxyl group and subsequent oxidation to a ketone (quinone/catechol equilibrium). The effect of scan rate (v) on the peak current (ip) and the peak potential (Ep) upon the electrochemical oxidation of both drugs were also examined. The slope values observed were close enough to the theoretically expected value of 0.5 for a purely diffusion-controlled current. The pH analyses revealed a linear dependence in the order of magnitude to that expected for a monoelectronic/monoprotonic reaction. To achieve unmistakable identification, differential pulse voltammetry was also used. The method uses the electrochemical oxidation of these molecules to produce an analytical signal that can be related to each compound concentration with an average lower limit of quantitation of 0.01mg/mL. The analytical identification for 25I-NBOH, 25I-NBOMe, and 2C-I was performed using the second oxidation wave, although the first oxidation wave was used in the quantification analysis.

A novel, fast, and sensitive electrochemical method for detection of 25I-NBOH using SCPE was achieved and all method characteristics demonstrated the method to be analytically valuable. The method is selective enough to identify the three compounds individually, even given the great similarity in their structure. The method is selective and achieved full differentiation between 25I-NBOH, 2C-I, and 25I-NBOMe.

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25I-NBOH, Electrochemical Identification, NPS

K17 Death From Poppy Tea Consumption

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After attending this presentation, attendees will understand the toxicological implications of home brewing poppy beverages and the biological concentrations associated with these types of deaths.

This presentation will impact the forensic science community by increasing awareness of the toxicity of this natural high by educating forensic investigators, pathologists, and postmortem toxicologists.

The historical practice of brewing poppy tea for its opioid-like effects is making a comeback with modern-day substance abusers. Whether it is brewed as an attempt to get high, as a source of natural pain relief, or to alleviate opioid withdrawal symptoms, the desired effects among users include euphoria, sedation, or the lessening of any negative symptoms of withdrawal, such as anxiety, nausea, and sweating.

This study presents three postmortem cases with opiate toxicology results that can serve as excellent case studies for debate on the hazards of poppy-drink ingestion. Enough attention has not been given to the dangers of this practice due to the variability of the morphine content of the opium exuded from the plant. While internet tea recipes offer guidance, differences in poppy seed/pod cultivation, washing, and infusing time are some of the reasons why beverages may contain different alkaloid concentrations from brew to brew. Variability in individual opioid tolerance in addition to other drugs also taken will impact the degree of toxicity of the opiates in the tea.

Free opiates (morphine, 6-acetylmorphine, codeine, hydrocodone, oxycodone, hydromorphone, and oxymorphone) in blood and urine are analyzed by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) following Solid Phase Extraction (SPE) using a method validated against the Scientific Working Group for Forensic Toxicology (SWGTOX) validation standard. The blood concentrations of free morphine and codeine were 0.94mg/L and 0.11mg/L in case one, 0.62mg/L and 0.034mg/L in case 2, and 0.16mg/L and 0.010mg/L in case 3, respectively. Urine was submitted to the laboratory for two of the three cases. The urine concentration of morphine and codeine in case 1 were 10mg/L and 0.98mg/L and in case 2 were 13mg/L and 1.7mg/L, respectively. None of these cases were positive for 6-acetylmorphine. The minor opium alkaloids thebaine and laudanosine were identified by routine basic drug extraction in the urine of cases 1 and 2.

A review of the scene evidence from all three cases will help practitioners understand the investigatory clues leading to probable poppy drink consumption.

Poppy Tea, Poppy Seed, Forensic Toxicology

K18 The Impact of Storage Temperature, Glucose, and Microorganisms on Blood Alcohol Concentration in Non-Decomposed Whole Blood

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After attending this presentation, attendees will have a better understanding of how storage temperature, time, and the presence of excess glucose, bacteria, fungi, and yeast have an impact on Blood Alcohol Content (BAC) in non-decomposed whole blood.

This presentation will impact the forensic science community by illustrating the effects of glucose and microorganism contamination on BAC over a six-month storage period.

The presence of microorganisms has been studied in biological matrices, particularly in urine, but to date there are no comprehensive, longitudinal studies that demonstrate the impact of microorganisms and excess glucose in whole blood. Current research has revealed that some bacteria and yeasts, particularly *Candida albicans*, can produce ethanol in blood samples through a fermentation pathway. Additionally, storage of samples in warmer temperatures can increase the bacteria's ethanol production.[1] Conversely, degradation of ethanol in blood alcohol samples can be caused by storage temperature, time of storage, and sample volume.[2] Currently, an international standard for the collection, handling, storage, and testing of blood alcohol samples has not been established. If samples need to be re-analyzed or significant time elapses between evidence receipt and analysis, the samples must be stored correctly to ensure accurate results.[3] This study illustrates the effects of sample volume, storage temperature, and presence or absence of excess glucose and microorganisms on BAC over a six-month period.

Two sets of stock solutions of seven different ethanol concentrations were prepared in defibrinated sheep's blood: 0g/dL, 0.05g/dL, 0.08g/dL, 0.10g/dL, 0.15g/dL, 0.20g/Dl, and 0.30g/dL. D-glucose was added to one set of stock solutions in sufficient quantity to result in a blood glucose measurement of at least 240mg/dL. The appropriate blood was then added to 10mL gray-stoppered BD Vacutainer™ blood collection tubes in varying amounts (2.5mL, 5mL, 7.5mL, and 10mL) by removing the stopper and adding the blood via a syringe. For each BAC, four groups of eight samples were prepared. Group 1 included tubes of each volume as described, with and without excess glucose stored at room temperature (25°C). Group 2 was stored refrigerated at 4°C. Groups 3 and 4 were inoculated with a mixture of *Saccharomyces cerevisiae, Candida albicans, Acinetobacter johnsonii, Fusarium oxysporum,* and *Staphylococcus aureus* to simulate microbe contamination by improper collection (these strains were chosen as some of the most abundant on skin and/or having been indicated as common sources of contamination).[4] All four sets were made at each BAC for monthly analysis (months 0-6), for a total of 1,568 tubes. Each month, samples were analyzed in duplicate with an internal standard of 0.005% 2-butanone in water by an Agilent® 7820AGC and 5977EMS with headspace after instrument calibration, with 0.10g/dL standards run every 24 vials. Additionally, samples from each BAC level were streaked on blood agar plates and incubated to determine viability of the bacteria, yeasts, and fungus. If excess glucose was present, tubes inoculated with the microorganism mixture produced ethanol. Refrigerated samples experienced less degradation than samples stored at room temperature. Sample volume affected the rate of decomposition; smaller sample volumes experienced greater amounts of sample degradation. At BAC greater than 0.20g/dL, the microorganism survival rate was lower.

The results of this study indicate that the presence of microorganisms, particularly in the presence of excess glucose, can negatively affect the accuracy of ethanol analysis and that storage conditions and sample collection conditions, if known, should be considered when analyzing BAC data obtained from casework.

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BAC, Contamination, Longitudinal

K19 An Analysis of Drugs and Their Metabolites in Saliva and Urine Using Various Swabs in Conjunction With Direct Analysis in Real-Time Mass Spectrometry (DART®-MS)

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After attending this presentation, attendees will be able to: (1) understand the application of DART®-MS to the analysis of illicit drugs and their metabolites on various swabs used in routine drug testing applications; (2) understand the pros and cons of toxicology analysis of buccal swabs and urinary swabs by DART®-MS; and, (3) understand the effects of swab composition, drug type, drug polarity, and collection time on the sensitivity of drug detection in biological samples.

This presentation will impact the forensic science community by improving examiner knowledge of the variables involved in the analysis of oral fluid and urine for the detection of drugs of abuse using DART®-MS as a detection technique for swab analysis.

Hypothesis: Application of DART®-MS is gaining momentum in the forensic sciences due to its fast analysis time and minimal sample preparation. In toxicology casework, various swabs may be used for oral fluid collection and analysis. Though not popular, urine may also be sampled by a swabbing technique after being donated by the testee to concentrate any drugs present in the urine onto the swab tip.[1-3] These swabs may vary in their performance based on the method used for identification of drugs on these swabs, the composition of the swabs (hydrophobicity/hydrophilicity of the fibers), the physical properties (solubility, acidity/basicity, etc.) of the drug, and other factors.

Methods: Positive ion mass spectra were acquired using a DART® ion source interfaced to an AccuTOF™ mass spectrometer. To test the utility of swab sampling techniques for the analysis of drugs by DART®-MS, four different types of swabs were used: CVS™ brand cotton swabs, 155C rayon swabs, and two polypropylene applicators, 4508C FLOQSwabs™ and 4504C FLOQSwabs™, with different tip shapes. Solutions of lidocaine, procaine HCl, diphenhydramine HCl, and quinine monohydrochloride dihydrate were prepared in concentrations ranging from 1mg/ml to 1ng/ml. Specificity, Limit of Detection (LOD), and Linear Dynamic Range (LDR) were established for each swab variant. Next, the method was applied to multiple illicit drugs and metabolites, including cocaine, Δ^9-tetrahydrocannabinol, benzoylecgonine, and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol. These drugs were used to determine the correlation between: (2) swab fiber polarity; (2) drug polarity; and, (3) sensitivity. These drugs were spiked into synthetic urine or synthetic oral fluid in concentrations ranging from 100ug/ml to 1ng/ml. Each swab was submerged in the solution for 3s and analyzed via DART®-MS.

Results: There was a positive correlation between swab fiber polarity, drug polarity, and sensitivity of drug analysis. When urine or oral fluid solutions of polar drugs were sampled with polar rayon or cotton swabs, sensitivity was an average of ~10X-20X compared to polypropylene applicators. When urine or oral fluid solutions of non-polar drugs were sampled with the non-polar polypropylene applicators, sensitivity was ~10X-20X lower. When polar drugs in urine or oral fluid were sampled by the swabs, peak intensities were greatest for non-polar swabs, followed by more polar swabs. Non-polar drugs gave the highest peak intensity when cotton swabs were used. The two polypropylene applicators are composed of the same fibers, but have different tip shapes; 4508C is slightly more rounded than 4504C, and 4508C always had a higher peak intensity versus 4504C, showing the impact that swab tip shape has on sensitivity. The application of these techniques to the analysis of various synthetic drugs will be presented.

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DART®-MS, Swabs, Forensic Toxicology

K20 The Concentration and Distribution of Methamphetamine (MA) and Amphetamine (AM) in MA-Related Postmortems

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The goal of this presentation is to inform attendees that concentrations of MA and AM in the blood could be estimated with the postmortem concentrations in Bile Juice (BJ), when Peripheral Blood (PB) or Cardiac Blood (CB) cannot be taken.

This presentation will impact the forensic science community by presenting a study that is expected to play a major role in estimating the postmortem concentration of MA and AM in MA-related death cases.

MA is a frequently abused drug in southern parts of South Korea due to the nature of the port area. Also, MA-related deaths often occur in this area more than in other areas of South Korea. This study compared the concentrations and distributions of MA and AM in MA-Related Postmortems (MRPs). Gastric content (GS), CB, PB, BJ, and urine were analyzed in 20 autopsy cases of MRPs.

A Forensic Toxicant Screening Test (FTST) for medicine, pesticides, and cyanide in GS, CB, and urine was conducted by Gas Chromatograph/Mass Spectrometry (GC/MS) or liquid chromatograph/tandem mass spectrometry.

A Forensic Drug Screening Test (FDST) for MA, delta-9-carboxytetrahydrocannabinol, cocaine, and benzodiazepine in urine was conducted by immunoassay, and ethyl alcohol screening in PB by GC. MA and AM, if detected by the FDST, were subsequently confirmed and quantified by re-extraction and re-analysis in CB, PB, and BJ by GC/MS.

The postmortem MA and AM concentrations (mg/L) ranged from 0.42-204.10 (average 18.46) and 0.001-8.70 (average 0.68) in CB (n=15), 0.11-194.40 (average 13.29) and 0.001-7.30 (average 0.54) in PB (n=15), and 0.09-149.40 (average 27.46) and 0.001-4.20 (average 0.83) in BJ (n=9). The ratios of CB to PB (n=12) for MA and AM were 0.79-6.59 (average 1.92) and 0.21-6.67 (average 1.87), BJ to PB (n=8) were 0.77-10.50 (average 4.62) and 0.58-12.67 (average 5.36).

These data suggest that the postmortem MA and AM concentrations in CB are approximately two times (average 1.92 and 1.87) higher than those in PB, and four to five times (average 4.62 and 5.36) higher in BJ. The CB and BJ to PB ratios less than five are consistent with little to no propensity for postmortem redistributions; these data demonstrate that MA and AM are unlikely to exhibit significant redistributions.

According to these detection ratios, the concentrations of MA and AM in the blood could be estimated with those in BJ of the postmortem, which cannot take PB or CB.

Therefore, this study expects to play a major role in estimating the postmortem concentration of MA and AM in MA-related death cases. Additionally, it could assist in or predict the interpretation of MA intoxications and redistributions for the MRPs.

Methamphetamine, Postmortem, Concentration

K21 The Effects of Kambo: The First Case of Sudden Death in Forensic Literature

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After attending this presentation, attendees will be able to describe the effects of Kambo as a concurrent cause of death in users with heart disease.

This presentation will impact the forensic science community by explaining Kambo's biological effects and the need to control its sale.

Kambo is a substance obtained from the skin secretions of a frog, *Phyllomedusa bicolor*. The secretions are used during a purification ritual common in some regions of South America. After the skin is burnt, the secretions are applied, causing a type of poisoning in a process not yet fully understood. No fatalities associated have yet been reported. In the case reported, the biological effects of Kambo with the potential to cause death were analyzed.

A 42-year-old man was found dead in his house. The external examination showed recent burns on the left arm. A wooden stick, approximately 10cm long and burnt on one end, was found near the body. A plastic box labeled "*Kambo Sticks*" was also found. From his medical history, it emerged that the man was a chronic consumer of this substance. There was no history of drug use nor a family history of sudden premature death or ischemic heart disease.

A toxicology analysis was performed on biological fluids for alcohol, prescription medication, and illicit drugs. A toxicological study was also conducted on the Kambo sticks and on biological fluids (blood and vitreous humor). The analysis consisted of reverse phase High-Performance Liquid Chromatography (HPLC) and mass spectrometry with TripleTOF® 5600+ System. Internal analytical standards were used for the research of dermorphin, deltorphin A, phyllocaerulein, phyllokinin, sauvagine.

The heart exhibited left ventricular concentric hypertrophy. There were also apparent petechiae on the subpleural area and a subconjunctival hemorrhage. Histological examination revealed moderate coronary artery disease with a reduction of approximately 65% of the left coronary branch by the atheroma. The brain displayed intraparenchymal microhemorrhages. The lungs had subpleural petechiae and bullous emphysema, with fibrotic thickening of the septal interstitium and sporadic micro-granulomas. The myocardium exhibited fragmented myofibrils and areas with marked inter-myofibrillar connective tissue.

The toxicological screening was negative for cannabinoids, opiates, cocaine metabolites, benzodiazepines, and ethanol. The investigations performed on the sticks found in the house demonstrated the presence of deltorphin A, phyllocaerulein, and phyllokinin; however, regarding biological fluids, deltorphin A was isolated exclusively in blood.

Kambo is comprised of a peptide mix. The peptides contain opioids including dermorphin and deltorphins, vasoactive molecules including phyllocaerulein, phyllomedusin, phyllokinin, sauvagine, and antimicrobials including dermaseptins. The peptides affect the body both at a central and peripheral level. The effects of each peptide are not yet fully understood. In the case reported, the man died suddenly after the substance was applied. In this case, as noted by the clinical data reported by the family doctor, the young man didn't appear to have any diseases or symptoms, and didn't take any medicine for treatment or other drugs intravenously. From the testimony of his mother, death happened approximately 30 minutes after the application of the drug. The autopsy revealed a left ventricular hypertrophy. By analyzing the action of these peptides on the body, it is possible to assume that the chronic consumption of some of the cardioactive peptides could lead to the development of left ventricular hypertrophy. Coronary artery disease was also found, most likely associated with the man's lifestyle (smoker and overweight). Certainly, the more likely effect would be hypotension. Death might therefore result from hypoperfusion of the heart which, in this case, could well be exacerbated by the increased left ventricular mass and moderate coronary artery disease. In a heart exhibiting moderate coronary artery disease and left ventricular hypertrophy, this generated an acute cardiac ischemia and consequent ventricular fibrillation. The following pressure peak generated was demonstrated by diffuse cerebral micro-hemorrhages, subpleural petechiae, and subconjunctival hemorrhage. The positive results of the toxicological investigation made it possible to state that the substance could be one of the concurrent causes of death. It is also important to consider that this substance can easily be ordered and obtained from many websites without any controls and/or prescriptions.

Forensic Science, Kambo, Sudden Death

K22 An Evaluation of Alcohol Concentrations in Samples Referred to the Forensic Laboratory in Baghdad

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After attending this presentation, attendees will be aware of the minor impact of the problem of alcohol drinking in causing and contributing to the cause of death in all autopsy cases in Baghdad. The goal of this paper is to detect and measure the concentration of alcohol, tackle this issue, and reveal its scope.

This presentation will impact the forensic science community by revealing the size of the problem of alcohol intake in contributing to or causing death.

Alcohol is one of the world's leading risk factors for morbidity, mortality, and disability. In 2012, 5.9% of all global deaths were attributed to alcohol and 5.1% of all global diseases and injuries were attributed to its use as well. Its effect was more pronounced from neuropsychiatric disorders.[1] Annually, 88,000 people die from alcohol-related causes in the United States and it is considered the fourth-leading preventable cause of death. It is blamed for 31% of all driving fatalities.[2]

Alcohol is also related to many crimes. In 2013-2014, 53% of violent crimes were committed under the effect of alcohol, including assaults, wounds, sexual offenses, homicides, criminal damages, theft, and robbery.[3]

This study was a prospective study within the first six-month period of 2015 on postmortem blood samples referred to the main forensic toxicology laboratory in the medicolegal directorate in Baghdad for the detection and measurement of alcohol; 5ml to 10ml of blood was withdrawn for each sample and a Headspace/Gas Chromatograph/Flame Ionization Detector (HS/GC/FID) from Agilent 7890A was used.[4]

A fifty-milligram percentage of alcohol was considered the cut-off point and every result above 50% was considered to be a positive sample. In general, traumatic death was predominant among all victims.[5] From the total 1,275 samples, only 112 (8.8%) were positive, with males more than five times more frequent than females. There was a significant relation to alcohol intake with traumatic causes of death, yet in only 13 victims were the concentrations fatal.

This study also revealed that traumatic causes of death decreased significantly with advancing age. Only 12 positive samples were attributed to natural causes of death. In those victims, alcohol probably factored with their diseases in precipitating death.

Alcohol drinking is a minor problem, as it was detected in a small group of all cases, yet its association with traumatic death was significantly higher than natural death and its consumption was more than five times higher in males. Only in a limited number of cases was the concentration fatal.

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Alcohol, Ethanol, Postmortem

K23 The Evaluation of Direct and Indirect Biomarkers of Ethanol Consumption: A Likelihood Ratio (LR) Approach to Identify Chronic Alcohol Misusers for Forensic Purposes

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After attending this presentation, attendees will understand how to interpret and evaluate the values of direct biomarkers of ethanol consumption to be detected in hair and keratin matrix for forensic purposes.

This presentation will impact the forensic science community by informing attendees that the LR models proved to be capable of significantly discriminating non-chronic from chronic alcohol consumers.

The determination of direct ethanol metabolites, such as Ethyl Glucuronide (EtG) and Fatty Acid Ethyl Esters (FAEEs), to be quantified in keratin matrix samples is indicated as the gold-standard approach to efficiently identify chronic alcohol drinkers.[1,2] Even if cut-off values have been established by the Society of Hair Testing (SoHT) to interpret EtG and FAEEs results, it has been documented that several confounding factors may alter the correlation between alcohol consumption and biomarkers' concentration in hair (e.g., cosmetic treatments).[3] As a consequence, the adoption of the traditional univariate interpretative approaches seems not to be the best option, as it may lead forensic experts and physicians to infer misleading conclusions, thus triggering the possibility of employing alternative multivariate data interpretation approaches.

Due to the fact that the LR models overcome the drawbacks of the traditional univariate approaches, as no cut-off values are involved during the process of evidence evaluation, several LR models have been developed and tested.[4] In the practice, LR values reveal the support to be delivered to the evaluated propositions and LR results can be expressed by means of verbal scales. An LR approach evaluates the Evidence (E) in case of two different, and mutually exclusive, hypotheses by examining the collected data. In the present case, the first hypothesis (H1) is that the individual under examination is a non-chronic alcohol consumer. Otherwise, the second hypothesis (H2) states that the examined subject is a chronic alcohol misuser. At the current stage, the collected data consists of direct (FAEEs, EtG) biomarkers of alcohol consumption from more than 150 scalp hair samples of different individuals, representing both chronic and non-chronic alcohol drinkers target categories. Different multivariate LR models have been evaluated and their ability to discriminate chronic alcohol misusers from non-chronic alcohol consumers were examined. The performance of each model was evaluated in terms of rates of correct classification (%) and empirical cross-entropy parameters. Since satisfactory reduction of information loss have been observed, together with correct classification rates close to 100%, LR validated models proved to be capable of discriminating non-chronic from chronic alcohol consumers. Similar results have been observed when employing further multivariate data analysis strategies such as Partial Least Squares Discriminant Analysis (PLSDA).[5]

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Chronic Alcohol Drinkers, Hair Samples, Likelihood Ratio

K24 2017 Novel Illicit Opioids: Trends and Toxicological Insights

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After attending this presentation, attendees will be able to describe the changes in novel illicit opioid positivity in forensic casework over a 6- to 12-month time period in 2017.

This presentation will impact the forensic science community by detailing trends in the use of novel illicit opioids in 2017, including change in positivity over time, user demographics, and quantitative data from casework, all of which underscore the need for comprehensive toxicological testing with a dynamic scope for novel opioids and vigilance by investigators, forensic scientists, and legislators.

Novel illicit opioids are a major component of the opioid epidemic that has become a major public health crisis. Increased misuse and diversion of pharmaceutical fentanyl in the early 2000s has given way to exponential growth since 2013 with the appearance of illicitly synthesized fentanyl and the introduction of additional novel illicit opioids, such as U-47700, furanyl fentanyl, and carfentanil, which have subsequently been identified in toxicological casework. Toxicological identification involved either Liquid Chromatography/Time Of Flight (LC/TOF) or fentanyl-based immunoassay screening and targeted confirmations using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) or a Gas Chromatography/Mass Spectrometry (GC/MS) database match.

Positivity data was obtained from toxicology casework (death investigation, impaired driving, and hospital admissions) in which the commonly encountered novel opioids furanyl fentanyl, U-47700, carfentanil, butyryl fentanyl, para-Fluoroisobutyryl Fentanyl (FIBF), and acryl fentanyl were detected in blood samples (Table 1). Percent positivity indicates the proportion, relative to the totals for these drugs encountered by month.

	January		**February**		**March**		**April**		**May**		**June**		**TOTAL**
	N	%Pos	N	%Pos	N	%Pos	N	%Pos	N	%Pos	N	%Pos	
Furanyl Fentanyl	122	41.4	178	49.3	164	39.0	114	39.6	120	33.7	90	28.1	**788**
U-47700	34	11.5	31	8.6	53	12.6	47	16.3	68	19.1	45	14.1	**278**
Carfentanil	79	26.8	51	14.1	31	7.4	46	16.0	51	14.3	73	22.8	**331**
Butyryl Fentanyl*	6	2.0	17	4.7	45	10.7	18	6.3	23	6.5	7	2.2	**116**
FIBF*	34	11.5	28	7.8	55	13.1	29	10.1	63	17.7	80	25.0	**289**
Acryl Fentanyl	20	6.8	56	15.5	72	17.1	34	11.8	31	8.7	25	7.8	**238**
TOTAL	**295**		**361**		**420**		**288**		**356**		**320**		**2040**

Table 1: *Change in positivity from January 2017 to June 2017.*

**%Pos = %Positivity for month specified. *Butyryl Fentanyl and FIBF are not differentiated from their isomers isobutyryl fentanyl and para-fluorobutyryl fentanyl, respectively.*

During the same time period, a total of 5,589 fentanyl cases were encountered, in addition to 2,458 cases containing the heroin metabolite 6-monoactylmorphine. Carfentanil, furanyl fentanyl, and U-47700 became popular in 2016 and maintained popularity in 2017. Positivity of furanyl fentanyl has been declining as positivity for acryl fentanyl and FIBF have been increasing from 6.8% to 7.8%, and 11.5% to 25%, respectively, in casework between January and June. Several other fentanyl variants are also being detected in toxicology casework to a lesser extent. 3-methyl fentanyl has continued to be seen (70+ detections total) in 2017, after re-appearing in 2016 following a roughly 30-year hiatus. There have also been sporadic detections of valeryl fentanyl (*n*=1), 4-methoxybutyryl fentanyl (*n*=1), and fluoro fentanyl analogs (*n*=20). An additional "U" series compound, U-49900, has been confirmed in two postmortem cases and both cases were also positive for Tetrahydrofuran Fentanyl (THF-F), a new fentanyl derivative first seen in 2017. Additionally, methoxyacetyl fentanyl (*n*=7) and cyclopropyl fentanyl (*n*=1) are being detected with increasing frequency as of July 2017.

These new substances challenge the forensic toxicology and chemistry communities because they pose all the same risks of routine opioids while going undetected. The data also illustrates the short cycle time of many of these drugs as they can come and go before a toxicology laboratory can develop and validate a method for their detection. In addition, the potency of these derivatives and correspondingly low concentrations in biological fluids challenge the capabilities of routine analytical methodology, forcing laboratories to seek new technologies to keep abreast of the trends.

Novel illicit opioids have been confirmed by the reporting laboratory in 42 states and Canada. Although certain states, especially the Northeast United States, have greater prevalence for certain compounds, they are not isolated to one part of the country. The forensic science community in general needs to be aware of the impact of novel illicit opioids.

Opioids, Novel Psychoactive Substances, Methoxyacetyl Fentanyl

K25 The Morphine in Your Pantry — Understanding the Overdose Risk of Home-Brewed Poppy Seed Tea

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After attending this presentation, attendees will understand the risks involved with consuming home-brewed poppy seed tea, including death. Attendees will learn that regardless of extraction conditions, it is possible to rinse opium alkaloids from poppy seed coats by home-brewing methods. In particular, attendees will learn that it is possible to rinse lethal amounts of morphine from unprocessed poppy seeds at home.

This presentation will impact the forensic science community by demonstrating the need for further consideration regarding the legality of purchasing unprocessed poppy seeds in bulk.

Morphine, codeine, and thebaine are naturally occurring opiates found in the latex of the opium poppy (*Papaver somniferum*). This latex is harvested worldwide for both licit and illicit opioid production. Poppy seeds are commonly harvested for baking, and opium alkaloids are transferred onto poppy seed coats during the harvesting process. Bulk, unprocessed poppy seeds can be purchased online with no current legal repercussions.

Recently, a medical examiner reported the case of a 24-year-old male who died of morphine intoxication with aspiration pneumonitis; however, neither licit nor illicit sources of morphine were found at the scene. It was suspected that the decedent ingested a lethal amount of morphine from home-brewed poppy seed tea. A 5-pound bag of poppy seeds and a 33-fluid-ounce bottle filled with seeds and water were found at the scene.

Due to the recent increase in poppy seed tea-related deaths, this research proposed that lethal amounts of morphine could be extracted from poppy seeds by home-brewing methods. Codeine concentrations were investigated because codeine is another opium alkaloid with analgesic properties, and codeine and morphine can have compounding effects when both drugs are taken simultaneously. Thebaine concentrations were investigated because, although it has no analgesic properties, its presence in biological specimens is indicative of poppy seed consumption.

No studies to date investigated opium alkaloid content that can be extracted from poppy seeds by home-brewing methods. For this reason, 22 samples of poppy seed products were purchased from online sources and extracted with four home-brewing methods representative of recipes found on drug user forums. Poppy teas were produced in room temperature and heated water, with and without lemon juice (acid) modifier over 10min with gentle agitation.

Extracts were analyzed in triplicate, and morphine, codeine, and thebaine were quantified in these extracts by liquid chromatography-tandem mass spectrometry using a validated analytical method. Precision and accuracy were 4.2%–7.7% and 92.3%–103.4%, respectively, with minimal matrix effects (95.1%–103.7%). Morphine, codeine, and thebaine concentrations were <1mg/kg–2,788mg/kg, <1mg/kg–247.6mg/kg, and <1mg/kg–124mg/kg, respectively. Many samples yielded morphine levels that would correlate to lethal concentrations if moderate volumes of tea were consumed. Yield varied between all extractions for all analytes in all samples.

Forensic Science, Forensic Toxicology, Poppy Seed Tea

K26 Carfentanil-Related Deaths in Wayne County, Michigan: Epidemiology and Toxicology

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After attending this presentation, attendees will be able to describe the toxicological and epidemiological aspects of a series of 129 carfentanil-related fatalities in Wayne County, which includes the city of Detroit, MI.

This presentation will impact the forensic science community by providing attendees with the carfentanil concentrations observed in medicolegal death cases related to the drug in addition to the demographic information associated with the decedents, which will assist with the interpretation of drug concentrations in future carfentanil deaths.

Introduction: Carfentanil, a synthetic opioid with an analgesic potency estimated to be 10,000 times that of morphine and that is approved for veterinary use in the sedation of large animals, was analytically confirmed in blood samples from 129 death investigation cases investigated by the Wayne County Medical Examiner's Office between August 2016 and June 2017. Details of the findings in these cases are presented and discussed.

Methods: All cases presented were submitted to NMS Labs in Willow Grove, PA, for comprehensive toxicological analysis by a Liquid Chromatography/Time Of Flight/Mass Spectrometry (LC/TOF/MS) screen for approximately 250 drugs and their metabolites, which includes carfentanil and several other emerging novel opioids in an additional secondary targeted accurate mass database. Confirmatory testing for carfentanil was performed by quantitative Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) with a quantitative reporting limit of 0.10ng/mL and limit of detection of 0.03ng/mL. Carfentanil-positive cases were reviewed for other drug findings, as well as demographic case data.

Results: Carfentanil concentrations ranged from 0.1ng/mL to 14ng/mL (*N*=127) with two cases <0.1ng/mL. The mean and median concentrations were 0.75ng/mL and 0.44ng/mL, respectively. It was found that 87% of cases had a carfentanil concentration less than or equal to 1.0ng/mL. There were only three cases in which carfentanil was the only drug detected. In 87.6% of cases, another opioid was detected, including morphine (57%), fentanyl (43%), 6-acetyl morphine (38%), furanyl fentanyl (28%), and U-47700 (12%). Other common findings were ethanol (50%), cocaine and/or benzoylecgonine (45%), cannabinoids (31%), and alprazolam (21%). Death locations were at home (37%), at a scene (32%), or in a hospital (31%). Naloxone was detected in 78% of hospital deaths compared to 17% at home and 7.3% at a scene. Decedent demographics were mostly male (73%) and White (63.5%). Black or African American (32.5%) and other (4%) were slightly underrepresented compared to overall Wayne County demographics (52% White, 31% Black or African American, 7% Other). Total cases peaked between November 2016 and February 2017, after which there was a sharp decline in deaths related to carfentanil. In one case, an antemortem whole blood specimen was collected at the hospital approximately 14 hours prior to death. The antemortem blood and postmortem femoral blood concentration was 0.93ng/mL and 0.34ng/mL, respectively. The half-life of carfentanil in female elands was reported to average 7.7 hours.[1] This single human case appeared to have a half-life of 8.1 hours. While there are certainly caveats to this extrapolation of kinetic data, it does suggest that a one-time dose of naloxone (half-life 64 +/- 12min) would be insufficient as an antagonist. To various degrees, all of the reported cases involved police, first responders, investigators, morgue assistants, and pathologists. There was not a single incident of any adverse effects to any individual in any case involving incidental exposure to carfentanil.

Conclusions: Carfentanil was detected as an opioid of abuse in 129 cases Wayne County, MI, between August 2016 and June 2017. Although it was detected with other opioids in more than 80% of cases, it contributed to the cause of death in all cases that were considered drug-related fatalities. The decrease in the incidence of carfentanil toward the end of the reporting period suggests the possibility of new novel opioids that may be contributing to drug-related deaths.

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Carfentanil, Opioids, Fatalities

K27 A Field Performance of the DrugTest 5000® and DDS®2 Onsite Oral Fluid (OF) Devices by Oregon and Vermont Drug Recognition Experts (DREs)

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After attending this presentation, attendees will be able to describe the field performance of the Draeger DrugTest 5000® (DT5000®) and Alere™ DDS®2 Onsite OF devices compared to Mass Spectrometry (MS) OF confirmatory tests and understand the usefulness of drug tests in Driving Under the Influence of Drugs (DUID) cases.

This presentation will impact the forensic science community by informing attendees regarding the use of onsite OF tests in identifying drug use.

Background: OF is easily collected and tested at the roadside to rapidly identify recent drug intake. Collection is non-invasive and gender neutral, with results available in minutes rather than hours for urine or invasive blood collection. During this delay, blood drug concentrations may decrease greatly, especially Δ9-Tetrahydrocannabinol (THC), hampering identification of recent drug consumption.

Methods: OF was collected with the DT5000® in Oregon ((OR), N=57) and Vermont ((VT), N=35), and the DDS®2 (N=23) in VT. Only one device was utilized per individual. Cutoff concentrations and performance for the combined OR and VT DT5000® data and the VT DDS®2 data are in the tables below. NMS Labs performed confirmation testing on OF collected with Immunalysis™ Quantisal® devices. All OR samples were collected in DUID cases, while 49 VT cases were from a court-ordered rapid intervention program and 9 from DUID cases.

Results:

DT5000 (OR & VT)

Drug, cutoff ng/mL	TP	FN	FP	TN	Sensitivity %	Specificity%	Efficiency %	PPV %	NPV %
THC, 5	47	1	0	44	97.9	100	98.9	100	97.8
Cocaine, 20	5	0	1	85	100	98.8	98.9	83.3	100
Amphetamine, 50	23	7	2	60	76.7	96.8	90.2	92	89.6
Methamphetamine, 35	34	0	2	56	100	96.6	97.8	94.4	100
Benzodiazepines, 15	2	0	0	90	100	100	100	100	100
Opiates, 20	31	1	3	56	96.9	94.9	95.6	91.2	98.2
Methadone, 20	3	0	0	89	100	100	100	100	100
Overall	145	9	7	480	94.2	98.6	96.9	95.4	98.2

DDS®2 (VT)

Drug, cutoff ng/mL	TP	FN	FP	TN	Sensitivity %	Specificity %	Efficiency %	PPV %	NPV %
THC, 25	3	2	0	15	60	100	90	100	88.2
Cocaine, 30	2	0	0	21	100	100	100	100	100
Amphetamine, 50	3	0	3	17	100	85	87	50	100
Methamphetamine, 50	0	0	0	23	n/a	100	100	n/a	100
Benzodiazepines, 20	0	0	0	23	n/a	100	100	n/a	100
Opiates, 40	3	1	1	18	75	94.7	91.3	75	94.7
Overall	11	3	4	117	78.6	96.7	94.8	73.3	97.5

Discussion: For the DT5000® device, sensitivity, specificity, and efficiency exceeded 94.9%, except for the amphetamine assay, which had 7 FN tests. This could have been due to the much lower 10ng/mL OF amphetamine confirmation test, and the 2 FP tests could have been due to cross-reactivity of the DT5000® antibodies with other sympathomimetic amines. For 641 OF samples and seven drug classes, the DT5000® had sensitivity, specificity, and efficiency of more than 94.2%, with high Positive Predictive Values (PPV) and Negative Predictive Values (NPV) of ≥95.4%. The DDS®2 device had 78.6% sensitivity, 96.7% specificity, and 94.8% efficiency, with a high NPV of 97.5% and a lower PPV of 73.3%. The poorer THC DT5000® results may be the result of a higher THC cutoff or that there were only five confirmed positive THC samples or that different individuals were tested. The amphetamine assay specificity was problematic with a PPV of only 50%. Although there were many cocaine tests for the DT5000® evaluation, there were too few positive cocaine, benzodiazepines, and methadone cases to draw conclusions about sensitivity, and for the DDS®2, there were only 11 positive cases in the entire set. For DUID cases, PPV is important because of the consequences on the driver from an FP test. There were only 1.1% FP tests for the DT5000® and 3.0% FP for the DDS®2. In drivers with negative field tests or when results are inconsistent with observed intoxication, supplemental tests should be ordered because the onsite OF devices test for a limited number of drug classes.

Conclusion: The devices achieved good specificity, with better sensitivity for the DT5000® as compared to the DDS®2 device. In addition, savings on cost of transport time, officer time, phlebotomist costs, and a reduction in the number of witnesses required for testimony may be substantial.

Oral Fluid, Onsite, DUID

K28 An Analysis of Ethanol in Blood and Oral Fluid Samples From Dosed Individuals by Headspace Gas Chromatography

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After attending this presentation, attendees will better understand the application of headspace gas chromatography to the determination of ethanol in blood and oral fluid. Attendees will also understand the relationship between ethanol concentrations in blood, oral fluid, and breath samples during a human dosing study.

This presentation will impact the forensic science community by providing evidence of a validated method for the analysis of both blood and oral fluid. The validated method was utilized in the analysis of samples from individuals dosed with ethanol in order to assess the viability of oral fluid as a matrix in Driving Under the Influence (DUI) applications. The use of headspace gas chromatography for this toxicological analysis allows for minimal sample preparation and little devaluation of the column with repeated usage.

Oral fluid has become a matrix of interest for forensic toxicological analysis, mainly for the qualitative and quantitative analysis of various drugs of abuse. Oral fluid is an ideal matrix for forensic analysis due to its lack of invasive collection procedure and ease of collection and monitoring, making the sample difficult to adulterate.[1-3] The findings of this study have the potential to impact current policy and forensic sample collection in suspected DUI cases. The use of oral fluid as a forensic specimen that is collected carside after probable cause of driving while impaired has been determined to have the potential to improve the quality and accuracy of the forensic toxicological analysis. The ability to collect oral fluid at the scene can potentially reduce the lag time between the traffic stop and obtaining the toxicological sample.

A method for the analysis of ethanol in blood and oral fluid was developed. In this study, a Perkin Elmer® HS-Clarus® 580 headspace gas chromatograph with two flame ionization detectors and a TurboMatrix™ 40 autosampler was utilized. A single headspace injection was split between two columns, Elite-BAC1 (30m x 0.32mm x 1.8µm) and Elite-BAC2 columns (30m x 0.32mm x 1.2µm). Helium carrier gas at a flow rate of 12.30mL/min was utilized, and the column temperature was set to 70°C. The method was validated using aqueous solutions, bovine blood, human blood, and human oral fluid with Lower Limit of Detection (LLOD) and Lower Limit of Quantification (LLOQ) values of 0.01% for ethanol. Calibration curves demonstrated good linearity for the BAC1 and BAC2 column where the r^2 values exceeded 0.999.

A controlled dosing study was performed utilizing subjects who consumed a pre-determined amount of wine (11.5%) to reach a target blood alcohol concentration of 0.05g/dL. Blood, breath, and oral fluid samples were collected from subjects prior to the consumption alcohol. Blood and breath samples were collected every 15 minutes over 3 hours; oral fluid samples were collected every 5 minutes for the first 30 minutes post-consumption and every 15 minutes following for 3 hours. Blood and oral fluid samples were prepared using 3mL of internal standard (0.016% *n*-propanol), 300µL of sample, and ¼ teaspoon of NaF/NaCl salt mix. Breath samples were measured with a portable breath-testing device. Results revealed the ethanol concentration profiles correlated well between blood and oral fluid. The Pearson correlation values between samples of oral fluid and blood were 0.92–0.97.

In conclusion, the validated method for the analysis of ethanol in blood and oral fluid samples illustrates the utility of oral fluid samples as a matrix in DUI investigations. The ease of collection of oral fluid and the fast and simple sample preparation for analysis makes this method viable for implementation in a forensic toxicology laboratory for analysis of DUI samples.

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Forensic Toxicology, Blood Alcohol, Oral Fluid

K29 Alcohol Extrapolations: Scientific, Legal, and Ethical Considerations

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After attending this presentation, attendees will better understand the various methods for performing alcohol extrapolation, issues with using only the Widmark formula, and the scientific and legal expectations when performing alcohol extrapolations.

This presentation will impact the forensic science community by discussing a more scientifically robust approach for performing alcohol extrapolations by accounting for individual differences in volume distribution and elimination rates, thus representing Blood Alcohol Concentration (BAC) extrapolations as a range of values rather than a single point estimate.

Forensic science laboratories are often faced with Driving Under the Influence of Drugs (DUID) and Drug-Facilitated Sexual Assault (DFSA) cases involving alcohol. Forensic toxicologists and the justice system are frequently faced with questions pertaining to the BAC, or level of intoxication, experienced by an individual at a particular time in which an incident occurred. Direct measurements of the blood are typically taken at a time after the incident occurred and forensic toxicologists are frequently tasked with the determination (estimation) of the actual BAC at the time the incident occurred using a combination of chemical analyses of the blood or breath and any of the various empirically derived extrapolation methods. Many experts attest to BAC extrapolations using the original Widmark factor for estimating volume distribution without consideration for successive improvements to the formulas by Watson, Forrest, Ulrich et al., and Seidl et al., as well as assumptions that all of the alcohol had been absorbed at the time of the incident and the individual exhibits an average rate of elimination.[1-5] These simplistic assumptions and use of a single coefficient by Widmark are likely due to perceptions of the complexity to employ the more complicated algorithms, which account for gender, age, weight, height, water content, and Body Mass Index (BMI) as well as typical variations in absorption and elimination rates.

While simplistic, the single-point BAC result derived by limiting the calculation to a single method does not reflect the entire range of possible values at the time of the incident. Limiting the calculation to an average of the physiological ranges without consideration of a bounded interval of possible BAC values does not address individual differences and, therefore, could present incomplete and potentially misleading information to a fact-finder when evaluating whether a specific individual's BAC was greater than a statutory level at a particular time prior to the direct measurements. A more scientifically robust approach to alcohol extrapolations by expressing the full range of possible BAC values not only does provide a more thorough representation of the BAC, it provides a standardized framework for evaluating results across different laboratories, and cases in which assumptions and input parameters may otherwise vary. In *State v. Read*, the courts ruled that evidence is inadmissible if unfairly prejudicial — if it has the capacity to skew the truth or prejudice the truth finding process itself.[6] In "The Admissibility of Novel Scientific Evidence: *Frye v. United States*, a Half Century Later," Giannelli states that "the major danger of scientific evidence is its potential to mislead a jury; an aura of scientific infallibility may shroud the evidence and thus mislead the jury to accept it without critical scrutiny."[7] In *State v. Fausto*, the court asserted that, "When a witness is sworn in, he or she most often swears to 'tell the truth, the whole truth, and nothing but the truth.' In other words, a witness may make a statement that is true, as far as it goes. Yet there is often more information known to the witness, which if provided, would tend to change the impact of the information already provided."[8]

A review of court rulings clearly demonstrates the expectation, albeit the requirement, for clear expert testimony that in no way misleads a jury. The ANSI-ASQ (American National Standards Institute-American Society of Quality) National Accreditation Board's (ANAB's) document, *The Guiding Principles of Professional Responsibility for Forensic Service Providers and Forensic Personnel*, also speaks to the use of clear communications and the presentation of expert testimony that is not misleading to the judge or jury.[9] This presentation will discuss the methods for performing alcohol extrapolation, issues with using only the Widmark formula, and scientific and legal expectations when performing alcohol extrapolations.

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Alcohol Extrapolations, Legal, Scientific Formula

K30 Quantification of Minor Blood Cannabinoids and Their Utility as Recent Cannabis Use Markers in Driving Under the Influence of Drugs (DUID) Investigation Cases

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After attending this presentation, attendees will be able to adopt and validate a Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method for quantitation of minor cannabinoids, including Cannabidiol (CBD), Cannabigerol (CBG), and Cannabinol (CBN). As CBG and CBN were reported as recent cannabis use markers, attendees will be able to apply the findings to improve the interpretation of recent cannabis use.

This presentation will impact the forensic science community by providing information on a quantitative method for minor cannabinoids as recent cannabis-use markers and their utility to aid in the interpretation of blood cannabinoid results in DUID cases.

It has been demonstrated that cannabinoid blood concentrations following smoking are not well correlated with effects because their concentrations are a function of various factors (dose, potency, route of administration, users' experience level, frequency of drug use, etc.), making interpretation of results challenging. Determination of the presence of minor cannabinoids in blood can add value to the interpretation by providing a time frame on last cannabis use.

This study describes a sensitive LC/MS/MS method for quantification of minor cannabinoids (CBD, CBG, and CBN). Cannabinoids were extracted from 100µL of whole blood using liquid-liquid extraction, separated in a two-dimensional LC system with an Agilent® Poroshell 120 PFP (4.6mm x 5mm; 2.7µm) as a guard column and an Agilent® Poroshell 120 Bonus RP (2.1mm x 50mm; 2.7µm) as an analytical column, in a 5min run time and detected by an AB SCIEX™ 6500 system with turbo ion spray operating in positive ion mode with scheduled mass spectrometric Multiple Reaction Monitoring (MRM). The method validation protocol was based on the Scientific Working Group for Forensic Toxicology (SWGTOX) guideline to include linearity, Limit Of Detection (LOD), Lower Limit Of Quantitation (LLOQ), precision and accuracy, interfering substances, extraction efficiency, matrix effect, stability, dilution of samples, matrix matching, and carryover.

The calibration for CBD, CBG, and CBN was linear from 0.1ng/mL to 50ng/mL. Minimum extraction efficiency and the maximum observed matrix effect were 97.4% and 2.8% suppression, respectively. The method also met validation criteria for precision and accuracy at the LLOQ, low and high controls, dilution of samples, matrix matching, interference, and carryover.

Between January and March 2017, NMS Labs received 2,787 cases for a basic DUID panel consisting of TEN common drugs of abuse. Cannabinoids were presumptively positive by Enzyme-Linked Immuno-Sorbent Assay (ELISA) in 52% (*n*=1,450) of cases and the presence of Δ^9-tetrahydrocannabinol (THC) was further confirmed in 1,202 cases (83%) by LC/MS/MS with a Reporting Limit (RL) 0.50ng/mL. Of those cases, 98 samples with positive THC at various concentrations were additionally tested for CBG, CBN and CBD using the method described. Table 1 summarizes these findings.

	CBG (ng/mL)	CBN (ng/mL)	CBD (ng/mL)
Mean (±SD) (ng/mL)	0.44 (±0.51)	0.28 (±0.15)	0.25 (±0.33)
Median (ng/mL)	0.26	0.23	0.12
Range (ng/mL)	0.10 – 3.3	0.10 – 0.74	0.10 – 1.1
% positive	74 (*n*=72)	67 (*n*=66)	10 (*n*=10)

Table 1. *Blood concentrations and positivity rates for CBG, CBN, and CBD*

To assess the correlation between concentrations of THC and three minor cannabinoids, the correlation coefficient (*r*) values were calculated and analyzed. The analysis demonstrated that both CBG and CBN have *r* values greater than Critical Values when *p*=0.05, suggesting a statistically significant positive correlation between THC and CBG as well as THC and CBN. The results from the same analysis between THC and CBD and CBG and CBN showed no correlation.

CBD had a significantly lower positivity rate compared to CBG and CBN. This, partly because of its short detection window and the various concentrations depending on the growth environment and strains, excluded CBD as a reliable recent cannabis use marker.

Using the incident and blood collection times provided in 42 cases with positive CBG and/or CBN at a RL of 0.1ng/mL, the calculated time difference (Δt) ranged from 0.033 to 5.4 hours. Of those cases, 11-hydroxy-THC (11-OH THC) was positive above RL of 1.0ng/mL in 39 cases (93%) with the mean and median concentrations of 5.1ng/mL and 3.9ng/mL, respectively (range: 1.4-22).

The detection windows were also evaluated for CBG and CBN at the previously studied RLs. Of 72 CBG-positive cases, a majority (>90%, *n*=65) were in between 0.1ng/mL and 1.0ng/mL; CBG was above 1.0ng/mL in seven cases with an average Δt of 1.1 hours (*n*=3; time information provided). Smoking higher doses of cannabis may explain the longer detection window for CBG in this study compared to the previously reported 0.5hr. Of 66 CBN-positive cases, six cases had CBN above 0.5ng/mL (RL 0.1ng/mL) with an average Δt of 0.78 hours (range; 0.68–1.13 hours, *n*=5; time information provided). This was consistent with the previous finding.

K31 A Study of an Active-State CB1 Receptor Model and JWH Synthetic Cannabinoids

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After attending this presentation, attendees will better understand the interactions between synthetic cannabinoids from the JWH family with an active-state CB1 receptor model.

This presentation will impact the forensic science community by contributing to the understanding of essential interactions between specific substituents of JWH synthetic cannabinoids with specific CB1 receptor residues through molecular modeling. The knowledge of these key interactions can help forensic chemists predict the structure of new and undiscovered families of synthetic cannabinoids.

Synthetic cannabinoids have emerged onto the drug scene as an alternative to illegal marijuana.[1] Like delta-9-tetrahydrocannabinol (THC), the main psychoactive ingredient in marijuana, synthetic cannabinoids interact with G-coupled protein receptors found in the brain, immune system, and peripheral organs.[2] There have been two cannabinoid receptors identified: CB1 and CB2. The binding of THC and synthetic cannabinoids to the CB1 receptors that are prevalent in the brain, activating the receptors, is believed to be the cause of the drugs' psychoactive effects. In the 1990s, John W. Huffman et al. developed a large series of synthetic cannabinoids. (These compounds were all given the name JWH-XXX, after Huffman.) Many JWH compounds have been found to have similar effects as THC, functioning as CB1 receptor agonists.[3] These JWH compounds are seen in many synthetic cannabinoid or "Spice" drugs and have become an important area of research in the forensic science community.

In this study, an active-state CB1 receptor model, prepared by the Doerksen lab, was used to compare the ligand-receptor interactions between the CB1 receptor, the JWH synthetic cannabinoid family, and the THC compound. This study was conducted using Schrödinger's Maestro molecular modeling software. Synthetic cannabinoids from the JWH family were selected based on their affinity to bind to the CB1 receptor. The docking of the ligands to the receptor took place after both the synthetic cannabinoid ligands and CB1 receptor model were prepared for docking and a grid of the active site was generated. In order to increase understanding of the interactions between cannabinoids and the CB1 receptor, parameters can be set to provide the five best possible poses, or positions, for the ligands. Once the ligands were docked to the CB1 receptor model, the interactions were thoroughly analyzed. The information collected from this study includes: (1) the amino acid residue interactions with the ligands and the bond distances of these interactions; (2) the docking score of each ligand and each pose; and, (3) estimated binding affinities. This study revealed: the specifics of the interactions, such as the presence of π-π stacking; which interacting residues are hydrophobic, charged, or polar; and whether solvent exposure was important for parts of the molecules.

Results from this study reveal which residue interactions with the CB1 receptor are important for the JWH compounds and how these interactions vary between compounds within this family. Identifying the key interactions between the synthetic cannabinoids and the CB1 receptor is a step toward a better understanding of the effects of these drugs, including toxicity and potential for abuse. The long-term goal is to develop a database and computer program to help predict new structures and different classes of synthetic cannabinoids that have not previously been identified. Future research will include studying all classes of synthetic cannabinoids and other synthetic drugs in addition to the metabolites of these substances.

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Synthetic Cannabinoid, CB1 Receptor, Molecular Modeling

K32 The Evaluation and Preservation of Urine Specimens in Forensic Toxicology

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After attending this presentation, attendees will better understand how the pH of urine changes over time, the effect temperature has on pH change, the need for a preservative in urine samples, and the recommended minimum concentration of preservative required to stabilize urine pH. This research will directly benefit stability studies conducted on urine samples, as well as benefiting forensic and clinical laboratories analyzing for illicit substances in urine.

This presentation will impact the forensic science community by providing urine pH stability for 200 days as well as urine pH stability at room temperature from a multitude of participants to illustrate how variable the matrix is. Attendees will also understand the effects of added buffers, as well as preservatives, in varying concentrations in order to maintain the pH of a urine sample. The results of this study will allow toxicologists to better understand why some of their extractions have failed and also allows urine samples to be stored for longer periods of time to decrease the number of samples canceled for matrix stability problems. These results were evaluated for clinical and postmortem situations so a standardized method could be applied to both fields.

Urine is a commonly encountered matrix when screening for illicit substances in Driving Under the Influence of Drugs (DUID) cases within forensic laboratories and for clinical testing within hospital laboratories. Once a sample is received, after a period of storage, it generally undergoes an extraction procedure to remove any interferences from the matrix prior to instrumental analysis. Solid phase extraction techniques require pretreatment of samples to achieve an appropriate pH so the analyte of interest is in the appropriate form. This is generally achieved by use of a buffer. If the sample is not in the proper form, poor or no recovery may be a result.

During method development for a range of cathinones, it was determined that the pH of urine was changing over time and affecting these processes. It was determined that as the time a sample remained at room temperature increased, so did the urinary pH. This was hypothesized to be due to the breakdown of urea and creatinine into ammoniated compounds. This hypothesis was tested using Nessler's reagent and the Jaffee test. Nessler's reagent was used to measure the amount of urea present in a sample by scanning all wavelengths and reporting the absorbance at a specific wavelength. The Jaffe test was conducted in the same manner, but this test measured the amount of creatinine present in a sample. Appropriate calibration curves were made and urine samples were monitored over time to determine how the levels of these two compounds changed.

The addition of an appropriate preservative or buffer that can be added to urine to stabilize the pH of the matrix was investigated to help decrease the number of failed extractions and increase the stability of drugs present in urine over time. Sodium fluoride at 0.2% and 2.5% weight by volume (w/v) were added to urine samples and the pH was monitored to see if this was appropriate. Buffers of varying molarity were also evaluated. Samples were monitored in duplicate when stored in both the refrigerator and at room temperature for a period of 200 days. It was found that sodium fluoride at 0.2% w/v helped to maintain the pH with the necessary pH range required for successful extraction for a period of 90 days. Urine samples containing sodium fluoride at 2.5% w/v were found to be significantly less stable than that of the 0.2% w/v, but more stable than that of unpreserved urine. Without the addition of the preservative, urine pH is only stable for approximately two weeks. With the implementation of this research into case work, laboratories have the potential to extend the viability of the matrix and decrease the number of specimens canceled due to matrix instability. This research also provides the potential for detecting illicit substances for longer periods of time because pH is not adding to the degradation.

Urine, pH, Preservative

K33 Novel Stimulants N-Ethyl Pentylone and Dibutylone: Case Reports, Quantitative Confirmation, and Metabolic Profile Determination

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After attending this presentation, attendees will be able to evaluate N-ethyl pentylone and dibutylone concentrations in postmortem and Driving Under the Influence of Drugs (DUID) cases. In addition, attendees will be able to describe the metabolic biotransformation of N-ethyl pentylone and dibutylone and identify the metabolites in toxicological casework.

This presentation will impact the forensic science community by characterizing biomarkers of two emerging stimulants and providing analytical data for use in qualitative and quantitative interpretation.

Novel stimulants, like other Novel Psychoactive Substances (NPS), have been subject to various chemical modifications resulting in the appearance of a rapid succession of novel substances. Information related to the metabolism of these substances is often limited due to the lack of *in vitro* and/or *in vivo* studies, or unreported identification in authentic human specimens. In addition, uncharacterized chromatographic retention times, lack of identified target ions, and undetermined recreational or toxic concentration ranges create challenges for analytical detection and toxicological interpretation.

N-ethyl pentylone and dibutylone have been identified as emerging stimulants in impaired driving and death investigation casework, as well as in recreational drug users. The metabolic pathways of neither have been previously characterized.

Separate *in vitro* incubations of N-ethyl pentylone and dibutylone were performed with pooled human liver microsomes in duplicate over three days. Biotransformations identified for N-ethyl pentylone included demethylenation, ketone reduction, and hydroxylation. Biotransformations identified for dibutylone included demethylenation, ketone reduction, hydroxylation, and N-demethylation, forming butylone. After characterization of *in vitro* metabolic pathways, *in vivo* verification of these metabolites was accomplished using authentic specimens from toxicological casework or drug user studies.

Blood specimens (*n*=20) were quantitatively analyzed for N-ethyl pentylone by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) from postmortem cases (*n*=12), DUID cases (*n*=5), and cases with unspecified history (*n*=3). The mean (± Standard Deviation (SD)), median, and range for N-ethyl pentylone concentrations are shown in Table 1. One postmortem blood specimen was positive for pentylone (200ng/mL), a species not identified as a metabolite during microsomal incubations. In total, five metabolites of N-ethyl pentylone were confirmed in these specimens.

	Postmortem Cases (*n*=12)	**DUID Cases (*n*=5)**	**Unspecified Cases (*n*=3)**	**Overall (*n*=20)**
Mean	247 (±259)	41 (±26)	813 (±618)	280 (±374)
Median	155	34	1,140	95
Range	12-833	21-87	100-1,200	12-1,200

Table 1: *N-ethyl pentylone concentrations (ng/mL).*

Blood (*n*=4), urine (*n*=3), and vitreous (*n*=1) specimens were quantitatively analyzed for dibutylone and butylone by LC/MS/MS. All specimens were analyzed from postmortem cases (*n*=4), with overlap in specimens collected from the same individual. Specific case concentrations are shown in Table 2. In total, five metabolites of dibutylone were confirmed in these specimens.

	Blood (*n*=4)		**Urine (*n*=3)**		**Vitreous (*n*=1)**	
	Dibutylone	**Butylone**	**Dibutylone**	**Butylone**	**Dibutylone**	Butylone
Case 1	383	130	3100	69	250	108
Case 2	<10	385	16500	3060	-	-
Case 3	61	<10	2140	149	-	-
Case 4	1400	600	-	-	-	-

Table 2*: Dibutylone and butylone concentrations (ng/mL).*

N-ethyl pentylone and dibutylone were detected in combination in blood specimens (*n*=5) from death investigation cases. In four cases, mean (±SD), median, and range for N-ethyl pentylone concentrations were 479 (±316), 545, and 38-790ng/mL, respectively, and for dibutylone were 18 (±14), 12, and 10-40ng/mL, respectively. One additional blood specimen was positive for N-ethyl pentylone at 50,000ng/mL and dibutylone at 14ng/mL. Butylone was quantitatively confirmed in only two cases, above the analytical threshold.

Additional NPS identified in these cases included methylone, dimethylone, ethylone, 4-fluoroamphetamine, 4-chloro-alpha-PVP, acryl fentanyl, tetrahydrofuranyl fentanyl, carfentanil, para-fluoroisobutyryl fentanyl, U-47700, and U-49900. Causes of death included drug overdose, homicide, suicide, and vehicular crash. Reports of suspected "Molly" and "bath salt" use were noted in two cases. Specimens originated from Pennsylvania, New Jersey, New York, Florida, Texas, Utah, Vermont, Illinois, Missouri, and the District of Columbia. The majority of individuals were male (86%).

A comprehensive analytical approach is necessary to confirm novel stimulants and NPS in biological specimens, as drugs are often found in combination. Specimen concentrations for novel stimulants can vary, as high as µg/mL; therefore, appropriate dynamic range, detection limits, and dilution capabilities should be assessed. Rapid identification of biomarkers can be useful in the determination of unique and/or common metabolites between related substances, possibly providing additional information about ingestion and prolonging detection windows.

N-Ethyl Pentylone, Dibutylone, Postmortem

K34 Pharmacokinetic and Pharmacodynamic Differences Between Paramethoxymethamphetamine (PMMA), Paramethoxyamphetamine (PMA), 3,4-Methylenedioxymethamphetamine (MDMA), and Amphetamine in a Mouse Model

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After attending this presentation, attendees will be able to describe the pharmacokinetics of PMMA and related drugs. Attendees will also recognize the differences in behavior the drugs induce in a mouse model.

This presentation will impact the forensic science community by adding pharmacokinetic and pharmacodynamics data for PMMA and related drugs in a mouse model.

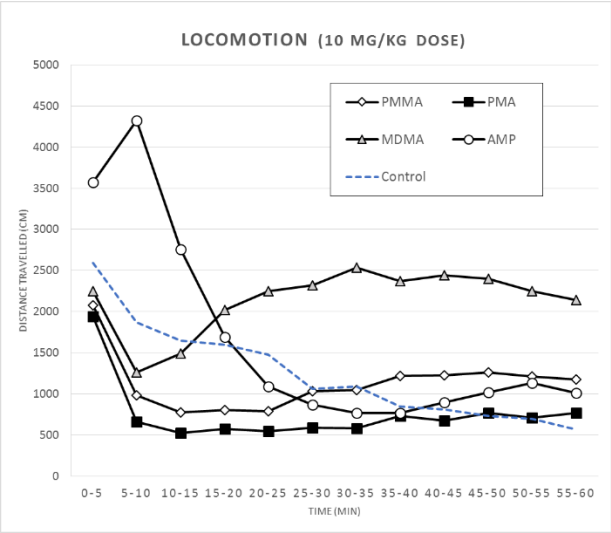
Background: Controlled study data regarding the pharmacology of PMMA and PMA in humans are lacking, but some data is available for the rat, pointing toward MDMA-like effects. Also, studies have suggested there is a delay in brain uptake that may trigger the user to take another dose because of absence of effect.

Goals: The goals of this study were to investigate the pharmacokinetics of PMMA and PMA in a mouse model and to compare their pharmacodynamics with MDMA and amphetamine.

Methods: The experiments were approved by the Animal Ethics Committee in Linköping, Sweden. Male C57/BL6 mice, 8-12 weeks old, weighing 25±1g were used for the experiments. The behavioral experiments were performed in an open field model. A video camera recorded the movements of the mouse during 60min and the movements were analyzed using EthoVision XT 9. Total distance travelled and time spent in the central zone were measured. In the behavior experiments, the animals (*N*=10) were dosed via intraperitoneal injection (i.p.) with either 0, 1, 5, or 10mg/kg of each substance immediately prior to the open field session.

Two pharmacokinetic experiments were conducted. First, dose concentration relationships were investigated using the same doses as in the behavior experiments with animals (*N*=5) sacrificed at 60 minutes. In addition, blood and brain kinetics were investigated for PMA and PMMA at 5mg/kg and 10mg/kg, respectively. The higher PMMA dose was chosen to increase the possibility of also measuring PMA formed from PMMA. Samples were obtained at 5, 10, 20, 40, 60, 80, and 120 minutes after injection.

Blood and brain concentrations of the substances were determined by Ultra High-Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS) using an AB Sciex™ 4500 coupled to a Shimadzu® LC-30AD liquid chromatograph. The column used was an Acquity® UPLC® BEH Phenyl (2.1mm x 50mm, 1.7µm). In brief, 100µL whole blood was fortified with internal standard, precipitated, then further diluted 10 times before analysis. The whole brain was weighed and homogenized in 0,075% HFo in acetonitril/ethanol (90:10), an aliquot fortified with internal standard and then diluted 20 times.



Results: There was a good positive correlation between dose and both blood and brain concentrations for all four substances with Pearson's *r* between 0.90 and 0.99.

The kinetics of PMA and PMMA were slightly different. PMMA distributed equally fast to blood and brain whereas PMA demonstrated a delay in maximum brain concentrations. Also, the disappearance of PMA from the brain was slower than for PMMA. The brain concentrations correlated well with the effects from the behavior experiments, with a longer duration of locomotor suppression for PMA. As can be seen in the figure, both PMA and PMMA resembled MDMA in their temporal pattern but with less pronounced effect, whereas amphetamine exhibited quite the opposite. The time spent in the center zone is a measure of anxiety. The only significant result was PMA at the 10mg/kg dose, which acted anxiolytic with more time spent in the center zone.

Conclusion: The findings suggest that the behavior effects are correlated to brain concentrations of PMMA and PMA and that the effects resembled those of MDMA, rather than amphetamine.

PMMA, Open Field, Pharmacology

K35 The Development of a High-Resolution Mass Spectrometry (HRMS) Library and Method Validation for Screening and Confirmation of 800+ Novel Psychoactive Substances (NPS) by Liquid Chromatography/Quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS)

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After attending this presentation, attendees will better understand the development of a compound database and spectral library containing more than 800 NPS, metabolites, and related compounds from a wide variety of drug classes with a particular focus on synthetic cannabinoids and stimulants. In addition, this presentation will demonstrate the use of standard mixtures rather than individual compounds to validate a comprehensive screening/confirmation method for the detection and identification of these NPS in biological fluids using LC/qTOF/MS.

This presentation will impact the forensic science community by presenting a developed HRMS library that can be used to help identify NPS.

NPS are of great interest to forensic toxicology labs due to their potentially high potency and ability to evade detection by many current screening methods. These compounds can also be rapidly developed to avoid current scheduling laws, causing a need to develop comprehensive detection methods covering a wide variety of drug classes.

An Agilent® 1290 Infinity® HPLC system and Agilent® 6530 QTOF-MS with Jet Stream technology Electrospray Ionization (ESI) source was used for this research. A total of 826 compounds to be included in the final method were analyzed using Flow Injection Analysis (FIA) to create an HRMS library with spectral data collected at three collision energies (10eV, 20eV, and 40eV) for each compound. Once the spectral data were collected, all compounds were run through an Agilent® ZORBAX® Rapid Resolution HD Eclipse® Plus C18 column (3.0mm x 100mm; 1.8µm particle size) to obtain retention times. LC was performed with a gradient of 95% A (5mM ammonium formate in HPLC water with 0.1% formic acid) and 5% B (methanol with 0.1% formic acid) from 0min–1min, increasing to 90% B over 1min–9.5min, then held at 90% B for the remainder of the 20min run. All retention times were used to create the final method for validation. The collected HRMS data and retention times were curated into a database/library using the MassHunter™ Personal Computer Database Library (PCDL) Manager software. Each compound entry also contained the following information: compound name, chemical formula, monoisotopic mass, chemical structure, and International Union of Pure and Applied Chemistry (IUPAC) name. Chemspider and Chemical Abstracts Service (CAS) numbers were also included, when available. The developed HRMS library is used to help identify NPS in real-time analysis, as well as to retrospectively search previously collected data.

In order to fully validate the method, calibration curves were created for each drug standard. Completing individual calibration curves for each of the 826 NPS included would be extremely time consuming and inefficient; therefore, an approach using a series of standard calibration mixes was investigated. Validation of the proposed method for 826 compounds involved the creation of 25 mixes containing between 29–37 different compounds each. The compounds selected for each mix were selected so that no compounds had the same retention time and had a minimum of 0.2min between compound peaks in the mix. Seven different calibration levels were chosen for method validation: 1, 2, 5, 10, 20, 50, and 100ng/mL. All calibrators also included an internal standard "supermix" made of 22 deuterated standards representing multiple NPS drug classes. Calibrations were performed with both methanol-based and spiked matrix (urine) mixtures for method optimization. For calibrations completed in urine, a simple "dilute and shoot" approach was used in which a 1:5 dilution was directly injected into the instrument.

To date, individual calibration curves have been created for nine different NPS mixtures representing nearly 300 of the 826 proposed NPS for the final validated method. LC chromatograms were analyzed using MassHunter™ QTOF Quantitation software. This approach was capable of identifying all compounds in each mixture. The results of these experiments clearly demonstrate the value of using standard mixes for method validation in comprehensive toxicological analysis in conjunction with an HRMS library. Work is continuing to create calibration curves for the remaining compounds using mixtures containing a maximum number of compounds to limit the number of mixtures needed for full validation.

LC/qTOF/MS, Novel Psychoactive Substances, Method Validation

K36 6-Monoacetylmorphine (6-MAM) Positivity: A Comparison of Two Methods

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After attending this presentation, attendees will better understand the impact of methodology and reporting limits on the ability to confirm heroin use through the detection of 6-MAM in forensic cases.

This presentation will impact the forensic science community by comparing the ability of two different methods to confirm heroin use through the evaluation of 6-MAM positivity.

With opiate use and abuse increasing, laboratories are under increased pressure to distinguish licit opiate use from illicit use; however, confirming the presence of heroin (6-diacetylmorphine) can be challenging due to the pharmacokinetics of the drug. Heroin is rapidly metabolized to 6-MAM and eventually to morphine. Confirming 6-MAM is essential to determining the presence of heroin in body fluids. Laboratory methodology and reporting limits can vary greatly in their ability to confirm and quantitate 6-MAM. This work compares the ability of a Gas Chromatography/Mass Spectrometry (GC/MS) method with a reporting limit of 10ng/mL and a Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method with a reporting limit of 1ng/mL to confirm and quantitate 6-MAM.

Method: Antemortem Driving Under the Influence of Drugs (DUID) and postmortem blood specimens submitted for opiate confirmation by GC/MS from April 2013 through April 2014 were reviewed. These results were compared to blood specimens submitted for opiate confirmation by LC/MS/MS from April 2014 through April 2015. Submissions in which 6-MAM confirmation was not performed for various reasons and cases that were none-detected for morphine were excluded. Percentages were rounded to the nearest whole number using conventional rounding rules. Statistical analysis was performed using student *t*-tests assuming unequal variance and that the populations were independent.

Results: A total of 14,932 cases that were submitted for opiate confirmation between April 2013 and April 2015 were included in this review. The overall 6-MAM positivity based on method increased from 15% by GC/MS to 32% by LC/MS/MS, an increase of 18% ($p < 0.05$). Submissions were also evaluated based on submission type: DUID (n=3,072) or death investigation (n=11,859). 6-MAM values ranged from 10ng/mL to 160ng/mL (mean 31ng/mL) by GC/MS confirmation and from 1ng/mL to 6,000ng/mL (mean 27ng/mL) by LC/MS/MS confirmation in DUID cases. 6-MAM positivity increased from 4% by GC/MS to 16% by LC/MS/MS, an increase of 12% ($p < 0.05$). Further investigation revealed that 87% of the DUID 6-MAM confirmations on LC/MS/MS were below 10ng/mL during this time period. Death investigation 6-MAM values ranged from 10ng/mL to 26,000ng/mL (mean 65ng/mL) on GC/MS and from 1ng/mL to 830ng/mL (mean 17ng/mL) on LC/MS/MS. 6-MAM positivity increased from 16% by GC/MS to 38% by LC/MS/MS, an increase of 22% ($p < 0.05$). In addition, 64% of the 6-MAM confirmations on the LC/MS/MS were below 10ng/mL in death investigations.

Conclusion: A comparison of these two methods demonstrated that a GC/MS method with a reporting limit of 10ng/mL could fail to confirm a large majority of heroin use in DUID and death investigation cases. In this particular review, a 6-MAM positivity rate increase of 18% was observed between the two methods.

Heroin, LC/MS/MS, GC/MS

K37 The Development and Validation of a Method for the Analysis of Novel Emerging Opioids

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After attending this presentation, attendees will be able to describe a Scientific Working Group for Forensic Toxicology (SWGTOX) - compliant approach to method validation for the analysis of designer opioid compounds in a variety of forensic samples using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) technology.

This presentation will impact the forensic science community by demonstrating the capability of an analytical method that can be used to simultaneously analyze 17 novel emerging opioid compounds, many of which have only recently appeared on the market.

In recent years, an increasing number of novel opioids have appeared on the illicit drug market and have been linked to the growing opioid crisis in the United States. In 2015, butyryl fentanyl was ranked in 12th place of the most frequently identified narcotic analgesics category in the National Forensic Laboratory Information System (NFLIS). By mid-year 2016, butyryl fentanyl was replaced by furanyl fentanyl, U-47700, and 3-methylfentanyl, which were ranked 12th, 13th , and 14th, respectively.

A method is described for the analysis of 19 of the most current novel opioid drugs in whole blood and serum, and 17 analytes in urine using LC/MS/MS. Blood and serum were analyzed quantitatively for butyryl fentanyl/isobutyryl fentanyl, MT-45, AH-7921, furanyl fentanyl, para-fluorofentanyl, ortho-fluorofentanyl, para-fluorobutyryl fentanyl/FIBF, 4-methoxybutyryl fentanyl, 4-ANPP, alpha-methyl fentanyl, 4-methylphenethyl acetyl fentanyl, U-47700, U-50488, acryl fentanyl, valeryl fentanyl, carfentanil, and beta-hydroxythiofentanyl. Urine samples were analyzed qualitatively for butyryl fentanyl/isobutyryl fentanyl, MT-45, AH-7921, furanyl fentanyl, para-fluorofentanyl, ortho-fluorofentanyl, para-fluorobutyryl fentanyl/FIBF, 4-methoxybutyryl fentanyl, 4-ANPP, alpha-methyl fentanyl, 4-methylphenethyl acetyl fentanyl, acryl fentanyl, valeryl fentanyl, carfentanil, and beta-hydroxythiofentanyl. The isomer pairs butyryl fentanyl/isobutyryl fentanyl and para-fluorobutyryl fentanyl/FIBF are not chromatographically separated in this method and are reported as a pair.

The method was validated according to a SWGTOX-compliant procedure, which for the quantitative portion evaluated precision and accuracy, limit of detection, lower limit of quantitation, linearity, stability in matrix and on-instrument, robustness, an evaluation of interfering compounds, matrix matching, dilution integrity, carry-over, matrix effect, and extraction efficiency. The validation for the qualitative portion of the method evaluated precision around the decision concentration (cut-off) stability in matrix and on-instrument, sensitivity and specificity, robustness, evaluation of interfering compounds, matrix effect, and extraction efficiency.

Sample preparation consisted of protein precipitation followed by solid phase extraction using Agilent® Plexa™ PCX 3mL/60mg extraction columns. The analytical method consisted of separation using a ZORBAX® RX-SIL (3mm x 100mm, 1.8 micron) column coupled with an Optimize EXP filter (0.2 micron) and a gradient elution utilizing ammonium formate, pH 4.0 (Mobile Phase A), acetonitrile (CH3CN), LC/MS grade (Mobile Phase B and weak wash), and formic acid in deionized water, 0.1% (strong wash). The analysis was performed on a Waters® ACQUITY® TQD MS/MS with a Waters® ACQUITY® Ultra Performance LC system.

This method produced data that met the acceptance criteria established for the validation. The quantitative portion of the analysis produced controls within 25% of target value, while the qualitative portion produced 94.1% sensitivity and 100% specificity during the validation. During the validation, it was determined that all analytes were stable in blood at room temperature for at least two weeks, and at refrigerated and frozen conditions for 30 days, with the exception of acryl fentanyl, which was only stable for one day at room temperature and one week refrigerated. In serum, it was determined that all analytes were stable at room temperature for at least two weeks, and 30 days refrigerated and frozen, except acryl fentanyl, which was stable for two days at room temperature, and MT-45, which was stable for one week at room temperature. In urine, it was determined that all analytes were stable for a minimum of one week at room temperature, and 30 days refrigerated and frozen.

Designer Opioids, Forensic Toxicology, LC/MS

K38 A Semi-Quantitative Retrospective Method Validation for Three Synthetic Cannabinoids With Analytical Confirmation in Toxicology Casework

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After attending this presentation, attendees will be able to discuss the importance of implementing analytical testing methods that allow for quick updates for continuously evolving synthetic cannabinoids and will be able to understand the benefits of performing a retrospective method validation to determine the concentrations of these analytes in biological fluids.

This presentation will impact the forensic science community by describing a retrospective method validation protocol used to determine semi-quantitative results of the synthetic cannabinoids 5F-AMB, 5F-ADB, and FUB-AMB, the most commonly seen analytes in toxicology casework from May 2016 through July 2017.

Since 2009, synthetic cannabinoids have presented a challenge to toxicology laboratories. As new compounds become available within the recreational drug market, labs are required to update their analytical methods to stay relevant. The development and validation of quantitative methods can be a long and demanding process, especially when there is a lack of deuterated internal standard for every analyte in the panel. Development of a qualitative confirmation method allows for faster incorporation of new compounds. Since these newer drugs are not part of many laboratories' routine testing procedures, there is limited information available on their expected levels in casework, making interpretation difficult; however, observed concentrations in biological fluids can help provide insight into the toxicity of these compounds. Therefore, a retrospective method validation of a qualitative method was performed for three compounds with a high positivity rate to acquire semi-quantitative data.

The qualitative assay was developed to detect 5F-AMB, 5F-ADB, and FUB-AMB, in addition to 24 related synthetic cannabinoids. During method development, it was noted that running a calibration curve improved the precision around the cut-off concentration; however, the quantitative controls for several analytes were not meeting the stringent requirements required by quantitative validations. Therefore, the method was validated qualitatively according to laboratory Standard Operating Procedure (SOP), including the evaluation of the cut-off concentration, sensitivity/specificity, carryover, matrix effect, interfering substances, and stability. Whole-blood samples were extracted using a liquid-liquid extraction, and analytes were detected using positive mode Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). Separation was achieved on a BEH C18, 2.1mm x 100mm column with mobile phases consisting of water containing 0.1% formic acid and an acetonitrile and methanol mixture (80:20).

A retrospective method validation was performed based on laboratory SOP and Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines. The Limits Of Quantitation (LOQ) were 25pg/mL for 5F-AMB and FUB-AMB and 50pg/mL for 5F-ADB. Linearity was established across two ranges (25-200pg/mL, and 50-400pg/mL) using five calibration points (n=5) with correlation coefficients ≥0.990 for all analytes and all back-calculated calibrators within 13% of target. The three synthetic cannabinoids were measured at three different concentrations for 15 separate days to provide acceptable between-run precision (13.5% Coefficient of Variation (CV)) and accuracy (±10.6%).

Based on the results of the retrospective method validation, all data that was still available on the laboratory instruments was reprocessed to determine the concentrations of 5F-AMB, 5F-ADB, and FUB-AMB. Data was included for all runs that met the following criteria: calibration curve correlation coefficients >0.990; back-calculated calibrators within ±20% of target and controls within ±20% of target. In this data set, there were 15, 97, and 112 cases above the limit of quantification for 5F-AMB, 5F-ADB, and FUB-AMB, respectively. Of these, 47% 5F-AMB, 24% of 5F-ADB, and 38% of FUB-AMB were below the reporting limit of the qualitative assay and thus had been reported "None Detected." The concentrations of all cases that fell within the calibration range are provided in the table below.

Analyte	Concentration (pg/mL)	# Cases >ULOQ
5F-AMB	68 ± 54 (n=10)	5
5F-ADB	200 ± 90 (n=52)	45
FUB-AMB	100 ± 60 (n=74)	38

The development of a qualitative method to detect synthetic cannabinoids allows for ease of updating the scope to remain relevant within the designer drug market. The ability to obtain semi-quantitative data through a retrospective method validation offers the forensic toxicology community information concerning the toxicity and expected concentrations of 5F-AMB, 5F-ADB, and FUB-AMB in whole blood samples.

Synthetic Cannabinoids, Retrospective Validation, Semi-Quantitation

K39 Fully Automated Detection and Quantification of Insulin Analogs by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) in Postmortem Vitreous Humor

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After attending this presentation, attendees will be informed about the current state of insulin analysis and the challenges surrounding its detection by LC/MS/MS in forensic samples. In addition, attendees will be able to implement a forensically validated LC/MS/MS method.

This presentation will impact the forensic science community by providing a novel approach for the simultaneous detection and quantification of human insulin and five pharmaceutical analogs and by describing its application in a series of forensic death investigations.

The analysis of biological specimens for the presence of exogenous insulin is of special interest in select postmortem investigations. Like other drugs and chemical agents, insulin may be implicated or suspected in the cause of a death; however, toxicological analysis is challenging due to complexities associated with immunoassay screening (cross-reactivity between endogenous and pharmaceutical analogs), challenges regarding multistage sample preparation (protein precipitation coupled to solid phase extraction or antibody immunopurification), as well as difficulties with mass analysis (poor fragmentation, low specificity transitions, analog/isotope coelution, and a reliance on low-flow microbore or nanobore chromatography). As a consequence, the determination of insulin in postmortem cases is not routinely performed. The work described here enables unambiguous differentiation of human insulin as well as five pharmaceutical analogs, including insulin glargine, glulisine, lispro, aspart, human, and detemir, through the use of robotic immuno-microchromatography coupled with insulin β-chain detection by LC/MS/MS.

Insulin extraction was performed on the Agilent® AssayMAP Bravo robotic platform using protein-G cartridges. Before extraction, 150µL of human vitreous humor is diluted 1:1 with Phosphate Buffer Saline (PBS) and fortified with porcine insulin as an internal standard. Cartridges are primed and conditioned with PBS prior to loading two mouse anti-insulin monoclonal antibodies (Santa Cruz SC-377071 and BioRad 5329-3806) to generate anti-insulin immunoaffinity microchromatography cartridges. Diluted vitreous humor is then loaded onto the immunoaffinity cartridges, washed sequentially with 4xPBS, 1xPBS, and 20% acetonitrile in 50mM ammonium bicarbonate, and eluted with 2% acetic acid into an existing volume of 40mM Tris(2-Carboxyethyl)Phosphine Hydrochloride (TCEP-HCL) in 30% acetonitrile. Following a brief incubation, insulin beta chains are analyzed in positive Multiple Reaction Monitoring (MRM) mode on an Agilent® 6495 triple quadrupole mass spectrometer coupled with a 1290 series Ultra High-Performance Liquid Chromatography (UHPLC). Chromatographic separation is performed using an Agilent® RRHD 300Å SB-C18 1.8µm, 2.1mm x 50mm analytical column with a stepwise gradient at 0.4mL/min over nine minutes.

Method validation was performed in accordance with the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines for Standard Practices for Method Validation in Forensic Toxicology. All analogs performed within criteria for acceptable performance. Parameters evaluated included linear range (500pg/mL–25,000pg/mL), limit of quantitation (500pg/mL), limit of detection (500pg/mL for insulin detemir and 125pg/mL for all other analytes), accuracy and precision (within and between run Coefficient of Variation (CV) <20%), interference, carryover, and stability (4°C and -20°C up to 30 days). In addition to the validation results, samples from five cases involving a suspected death by insulin have been analyzed. Of these, one case was positive for insulin aspart (743pg/mL) and one for insulin lispro (2,003pg/mL). A summary of the case history as well as an interpretation of findings will be discussed.

Insulin, LC/MS/MS, Overdose

K40 The Effect of Sample Preparation Techniques on Matrix Effects and Absolute Recovery of Opiates in Liver Tissue Using Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS): Part 1

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After attending this presentation, attendees will better understand the effectiveness of the various sample preparation techniques for the extraction of opiates from liver tissue in order to determine which method may be suitable for their own implementation.

This presentation will impact the forensic science community by increasing knowledge regarding sample preparation techniques for the forensic pathology and postmortem toxicology communities. Many sample preparation techniques are primarily designed for the extraction of drugs from blood or urine, and the adoption of these techniques for difficult matrices, such as liver tissue, has occurred without complete understanding of the effects of the matrix on the analysis of the analyte(s) of interest.

In this presentation, an evaluation of the effect of sample preparation techniques on matrix effects and absolute recovery of opiates in liver tissue will be presented. In postmortem toxicology, the concentration of drugs in the blood is often used to assist in the determination of the cause and the manner of death; however, central cavity drug blood concentrations can be unreliable because of the phenomenon of postmortem redistribution. This can be combated by evaluating liver concentrations, as drug concentration in liver is fairly stable after death. While liver is a valuable tissue for postmortem toxicology, the protein, fat, and phospholipid components of the matrix can interfere with analysis, and thus the drug must be isolated from the matrix prior to analysis. If proper and effective sample preparation and clean-up are not performed, matrix effects, such as ion enhancement or suppression, can hinder analysis and affect recovery of the drug. To limit matrix effects, it is necessary that the preparation technique used has the ability to extract the analyte as completely as possible while limiting the extraction of any interfering compounds.

There are many different approaches to sample preparation for drug extraction. The three traditional types of techniques are Solid-Phase Extraction (SPE), Liquid-Liquid Extraction (LLE), and filtration. A growing number of simple and rapid sample preparation techniques have become commercially available in recent years. These new techniques are commonly based on the traditional techniques but have added features to improve the extraction process. While these newer techniques have the ability to make sample preparation both easier and faster, there are still limitations. A majority of the user guides and technical notes for these new products focus on either blood or urine sample matrices. There is limited published data regarding tissue matrices, such as liver. For these techniques to be effectively used for liver samples, the matrix effects, absolute recovery, and process efficiency for extractions from liver must be evaluated.

These sample preparation techniques were evaluated for matrix effects and recovery by extracting opiates from homogenized liver tissue. Liver tissue was homogenized in saline at a ratio of 1:4. Homogenates were fortified with six opiates, at two concentrations (n=6), and their respective isotopic derivatives. The opiates analyzed were codeine, hydrocodone, hydromorphone, morphine, oxycodone, and oxymorphone. Three sets of samples were analyzed: neat, fortified before, and fortified after. Sample preparation was performed following manufacturer's guidelines (Waters® Oasis™ PriME HLB cartridge, Biotage® ISOLUTE® SLE+, and Biotage® ISOLUTE® PLD+) and using a laboratory validated LLE technique. Samples were analyzed using a previously validated UPLC-MS/MS method.

Results varied greatly between the methods evaluated. For Waters® Oasis™ PriME HLB, the observed matrix effects were between -35% and +5%, and recoveries were between 100% and 122%. For Biotage® ISOLUTE® SLE+, the observed matrix effects were between -18% and 0%, and recoveries were between 86% and 109%, with the exception of morphine, which had recoveries between 30% and 32%. For Biotage® ISOLUTE® PLD+, the observed matrix effects were between -16% and +50%, and recoveries were between 55% and 94%. For LLE, the observed matrix effects were between -59% to -37%, and recoveries were between 39% and 82%.

Liver is a difficult matrix to analyze. Sample preparation is not as simple as it is for blood or urine. It was observed that not all sample preparation techniques are effective or reliable for the extraction of opiates from liver tissue. Of the techniques evaluated, the Biotage® ISOLUTE® SLE+ was more effective at removing matrix effects and improved recovery.

Opiates, Liver, Sample Preparation

K41 The Identification of Adulterants in Preliminary Drug Analysis

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After attending this presentation, attendees will understand the implications of drug adulteration for urinalysis and how commercially available and common household adulterants can affect results in preliminary drug screening techniques, such as Enzyme-Linked Immunosorbent Assay (ELISA).

This presentation will impact the forensic science community by demonstrating how these immunoassay-based screening techniques are prone to producing false positive and negative results in the presence of adulterants. Adulteration of urine samples can circumvent current preliminary screening protocols (i.e., ELISA) and even common adulterant test strips. These results may affect criminal proceedings that are reliant on drug tests to determine convictions, compliance in probation, and court-based treatments.[1]

Despite drug abuse being one of the major issues that has plagued society for centuries, the technology to detect drugs and their metabolites in bodily fluids has only been accessible for less than 50 years.[2] Drug testing always begins with a screening technique in the form of immunoassays, such as ELISA, and adulterant testing strips may also be utilized to ensure the sample has not been manipulated.[3] Although response accuracy of immunoassays have increased drastically over the years, they only remain accurate approximately 95% of the time for the detection of drugs of abuse and their corresponding metabolites in urine samples.[4] This value decreases when samples have been adulterated.[2]

Approximately 30 urine samples were collected from anonymous volunteers. Each participant was required to complete surveys detailing the frequency of their drug use in the week prior to providing a sample. Based on this information, samples that may contain significant concentrations of common drugs of abuse and their metabolites were identified (i.e., THC, cocaine, amphetamine, and benzodiazepines). Aliquots of these urine samples were adulterated at different levels (i.e., 5, 10, 25, and 50% volume/volume (v/v)) with common and commercially available adulterants, including bleach, vinegar, eye drops, Drano®, nitrite, table salt, hydrogen peroxide, and hand sanitizer. Preliminary research using ELISA revealed that some adulterants (e.g., bleach, eye drops, Drano®) drastically reduce the concentrations of detectable drugs/metabolites in comparison to the unadulterated urine samples.

Adulterant test strips were also utilized to determine if, and at what level, they were able to detect when a urine sample had been tainted. Preliminary data revealed that most of the adulterants were not able to be detected at concentrations less than or equal to 10% v/v. Eye drops, specifically those that contain benzalkonium chloride, were not detected in the adulterated urine samples, even at high levels. This is of great concern, considering that eye drops drastically reduced the detection of THC, cocaine, and amphetamine by ELISA. These results suggest that new pre-screening techniques may need to be identified to combat and detect the presence of adulterants in urinalysis.

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ELISA, Adulterant Test Strips, Adulterants

K42 Using Medical Examiner Case Narratives to Improve Opioid Overdose Surveillance

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After attending this presentation, attendees will appreciate how using additional sources of information to classify opioid overdose cases can result in increases in the number of heroin-related classified deaths and in the identification of non-heroin, injection-related opioid analgesic deaths.

This presentation will impact the forensic science community by offering new tools for the characterization of opioid overdose deaths and the identification of meaningful subgroups of opioid users who can be targeted by public health programming.

Opioid overdose is a leading cause of death in the United States. While opioid analgesics were responsible for the rapid increase in overdose mortality during the 2000s, deaths due to heroin and fentanyl have increased sharply in recent years. Surveillance of the national opioid overdose epidemic demonstrate that the types of opioids causing overdose are evolving. For example, fentanyl mixed with or sold as heroin or other prescription opioids increases the risk of overdose death and complicates interpretation of postmortem toxicology. Opioid overdose surveillance systems report rates and counts classified by the type of opioid involved. Opioid types are extracted from the International Classification of Diseases (ICD) codes on death certificates, which may result in under-estimation or misclassification of specific opioid types.[1] Up to one-quarter of death certificates with drug overdose listed as the cause of death do not include the specific drugs implicated. Failure to include opioid type can result in substantially underestimated counts of overdose deaths involving opioid analgesics. Current opioid overdose mortality surveillance methods do not capture the complexity of the overdose epidemic. Most rely on death certificates which may underestimate heroin overdose deaths. In addition, categorizing deaths using characteristics beyond the type of opioid implicated in the overdose, such as the route of administration, can provide information to design and evaluate targeted public health interventions.

Methods: This study reviewed California Electronic Death Reporting System designations of cause of death and San Francisco Office of the Chief Medical Examiner postmortem toxicology reports and investigative case narratives for all unintentional deaths attributed to opioids occurring in the county of San Francisco from 2006 to 2012. Using these data sources, this study created enhanced classification systems for heroin-related and injection-related opioid overdose deaths and compared demographic, death scene, and postmortem toxicology characteristics between these groups.

Results: This retrospective analysis resulted in the identification of 816 unintentional opioid overdose deaths during the time period of interest. This study classified 152 of these deaths (19%) as "standard" heroin deaths (designated by the case medical examiner or confirmed by the presence of 6–monoacetylmorphine). An "expanded" classification of heroin deaths using data from postmortem toxicology reports and case narratives added 20 additional heroin deaths (+13% increase), accounting for 21% of all opioid deaths. Based on case narratives, 205 deaths (25%) were injection-related, 60% of which were attributed to heroin. A combined classification of enhanced heroin and injection-related deaths accounted for 31% of opioid overdose deaths during this period.

Conclusions: Using additional sources of information to classify opioid overdose cases resulted in a modest increase in the count of heroin-related deaths, but identified a substantial number of non-heroin injection-related opioid analgesic deaths that would otherwise have gone amiss. This current study reveals that including the route of administration in the characterization of opioid overdose deaths will identify meaningful subgroups of opioid users who can be targeted by public health programming.

This study was supported by a National Institute on Drug Abuse (NIDA) grant.

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Opioid Overdose, Injection Drug Use, Heroin

K43 Prescription Drug Degradation in a Simulated Postmortem Blood Model

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The goal of this presentation is to demonstrate the stability of prescription medications in an environment of optimal microbial activity, such as may be encountered in decomposed postmortem specimens.

This presentation will impact the forensic science community by providing fundamental information in regard to the stability of two important classes of drugs during the postmortem interval and throughout all stages of the analytical process. This information will assist attendees in the interpretation of antidepressant and antipsychotic drug concentrations measured in postmortem specimens.

Hypothesis/Proposition: Degradation of xenobiotics by micro-organisms is a complication that must be accounted for by forensic toxicologists analyzing postmortem specimens. This phenomenon is especially of concern prior to specimen collection, as during the postmortem interval (between death and autopsy), environmental conditions may favor microbial activity. Prescription medications (e.g., antidepressants and antipsychotics) are commonly observed in casework. Therefore, it is important to establish whether medications can degrade during this period to ensure accurate quantitation and detection of degradation products in toxicology screening methods. This study utilizes a "simulated postmortem blood" model enriched with microorganisms to investigate prescription drug stability.

Methods: The "simulated postmortem blood" model was constructed by directly inoculating antemortem blood (sourced from the Australian Red Cross Blood Service) with microorganisms from pooled stool samples of nine healthy donors. Antipsychotics investigated were in the structural classes of phenothiazines, tricyclics, thioxanthenes, butyrophenones, phenylpiperazines, and benzo(iso)thiazolepiperazines. Antidepressants investigated were tricyclics, Norepinephrine Reuptake Inhibitors (NRIs), and Noradrenergic and Specific Serotonergic Antidepressants (NaSSAs). These drugs were spiked into the model samples and non-inoculated controls. Risperidone was included in all experiments as a known microbially labile "positive" control. Preserved samples with 2% weight by volume (w/v) sodium fluoride were also prepared concurrently for both the model and non-inoculated controls. An Agilent® 1100 Series LC-UV was used to quantitatively monitor drug degradation over the course of a week's incubation of the samples at 37°C and extended incubations at room temperature, 4°C, and -20°C. Microbial communities were profiled throughout the experiments to determine which species were present in the initial inoculations and how communities changed over time with respect to sample environment, drugs present, temperature, and the presence of preservatives.

Results: Successful inoculation of viable microorganisms from the pooled stool samples was confirmed by the degradation of risperidone to its established bacterial degradation product, 2-hydroxybenzoylrisperidone, in unpreserved "simulated postmortem blood" samples. No degradation of risperidone was observed in the non-inoculated controls, which was consistent with prior studies performed to assess its stability in blood. After a week at 37°C, minimal losses were reported for all other investigated antipsychotics with none exhibiting significantly enhanced degradation in the "simulated postmortem blood" samples compared to the non-inoculated antemortem blood controls. In non-inoculated unpreserved samples, losses of up to 50% were observed for the phenothiazine antipsychotics after 38 days at 37°C. Experiments are currently ongoing for antidepressant drugs and extended incubation samples. At the time of presentation at the AAFS 2018 Annual Scientific Meeting, antipsychotic drugs will have been incubated at room temperature, 4°C, and -20°C for seven months. Results and analysis of microbial communities for 37°C experiments will also be completed later in 2017.

Conclusion: The simulated postmortem blood model allowed for the investigation of drug degradation as caused by a wide variety of relevant microorganisms; however, microorganisms sourced from unhealthy individuals, those taking any drugs or medications, and invasive species that may enter the body after death were not targeted in this study. Therefore, the potential for the postmortem degradation of these drugs cannot be excluded in all cases.

Drug Degradation, Putrefaction, Prescription Drugs

K44 Strange Bedfellows: Fentanyl Mixed With the Antiquated Poison Strychnine

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After attending this presentation, attendees will be familiar with two overdose deaths in which toxicological analysis detected strychnine in addition to fentanyl. These cases highlight the diverse methods often employed by clandestine laboratories in the production of illicit drugs.

This presentation will impact the forensic science community by providing two examples of cases of fentanyl overdoses in which strychnine was also detected. Attendees will find it an interesting juxtaposition of a modern synthetic drug with an antiquated natural poison. These cases also emphasize the wide spectrum of drugs the modern toxicologist could encounter.

The potent synthetic opioid fentanyl was first developed in the 1960s, having been derived from the structurally related drug meperidine. After discovering its potent effects, fentanyl was and is used regularly for palliative purposes. Illicit use of fentanyl began in the 1970s and continues to grow. Like all opioids, overdose deaths related to fentanyl have also increased dramatically in recent years. This growing demand has, in turn, been met by an increased supply and has also spurred greater diversity in techniques employed by clandestine laboratories in the illicit preparation of fentanyl. Presented here are two unusual death cases in which it is proposed that the illicit fentanyl was prepared with strychnine.

A 59-year-old White male was complaining of heartburn and vomiting one evening and was later found dead in his bed. Autopsy revealed changes related to hypertension, but no specific anatomic cause for death. Toxicological analyses of iliac blood reported fentanyl (0.003mg/L), strychnine (<0.025mg/L) and heroin metabolites: 6-monoacetylmorphine (0.006mg/L), morphine (0.159mg/L), and codeine (0.011mg/L). Death was attributed to heroin and fentanyl toxicity. Four days later, a 29-year-old Black male in another city, but in the same county, was discovered dead on his living room floor. The decedent had recently been released from prison and had also been treated at a hospital for pneumonia. Autopsy did not detect any residual pneumonia in the lungs or other anatomic cause for death. Toxicological analyses of iliac blood detected fentanyl (0.008mg/L), olanzapine (<0.025mg/L), and strychnine (<0.025mg/L). Death was attributed to fentanyl toxicity.

Strychnine is a potent alkaloid classically derived from the seeds of the *Strychnos nux-vomica* tree, which grows in warm climates in southern Asia and Australia. Strychnine was first isolated from the *Strychnos* genus in 1753, though the toxic effects of the *nux-vomica* bean had been well known in India and China for centuries before that. It is primarily used as a pesticide to kill small vertebrate pests such as rodents and has restricted availability in the United States due to the potential for deaths of animals for which it is not intended. Consumption of strychnine causes generalized muscle spasms. At lower doses, this can be restricted to tachycardia, cramping, rigidity, and agitation. Higher doses can lead to seizures, hypertension, cyanosis, and opisthotonus (dramatic back spasms causing arching of the back and neck). Death can occur from resultant cardiac arrest, respiratory failure, or brain damage.

In neither of these two cases was strychnine determined to be at sufficient levels to have contributed to death, especially given the presence and concentrations of the opioids. Nonetheless, its presence in these two cases underscores the diverse methods employed by clandestine laboratories in the production of illicit opioids and also illustrates an unusual marriage of a historic natural poison with more modern, synthetic drugs such as fentanyl.

Fentanyl, Strychnine, Opioids

K45 An Investigation Into the Analysis of Fentanyl in Postmortem Blood Using Biocompatible Solid-Phase Microextraction (BioSPME).

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After attending this presentation, attendees will better understand how BioSPME can be an alternative extraction method for fentanyl in postmortem blood.

This presentation will impact the forensic science community by providing an extraction method that is faster than current analytical methods. BioSPME coupled with Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) allowed for minimal sample collection, preparation, and shorter analysis time in the analysis of postmortem blood samples from overdose victims.

The abuse of opioids, particularly fentanyl, has become a slow-motion mass disaster over recent years in the United States. Due to the frequent abuse of opioids, there has been an increase in drug-related deaths.[1] Forensic pathologists are responsible for collecting various postmortem samples that are then sent to a toxicology laboratory to be analyzed for drugs, such as fentanyl. This process can be time consuming and may result in a backlog, which could hinder a criminal investigation. A solution could be BioSPME using coated fibers that can be directly injected into a biological matrix and absorb any drug present without the interference of macromolecules, thus allowing for a faster analysis time.

A method has been developed to analyze fentanyl in postmortem blood using BioSPME followed by GC/MS and LC/MS/MS analysis. BioSPME fibers were conditioned, directly injected into blood, washed, filtered, desorbed into solution, dried down, and reconstituted. The extracted samples were screened by GC/MS and subsequently analyzed by LC/MS/MS. GC/MS was performed using splitless injection on a Rxi-5Sil MS column (30.0m x 0.25mm, 0.25μm) in the Selected Ion Monitoring (SIM) mode. Samples were confirmed using an AB SCIEX™ 3200 QTRAP® triple quadrupole MS with an Electrospray Ionization (ESI) source in the positive ion mode. LC was performed on a Shimadzu® LC system using an Ascentis® Express Biphenyl column (50mm x 2.1mm, 2.7µm) with the weak mobile phase of 0.1% (volume/volume (v/v)) formic acid in water and the strong mobile phase of 0.1% (v/v) formic acid in acetonitrile. The flow rate was 0.30mL/min, column temperature was 30ºC, injection volume was 1μL, and an analysis time of seven minutes per sample. This method was developed using bovine blood, then applied to 43 postmortem blood samples from overdose victims from the Lehigh County Coroner's Office in Allentown, PA.

In conclusion, the use of BioSPME as an extraction method allows for minimal sample preparation and collection for the detection of fentanyl in postmortem blood.

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1. U.S. Drug Enforcement Administration, Office of Diversion Control. National Forensic Laboratory Information System Special Report: Opiates and Related Drugs Reported in NFLIS, 2009-2014. Springfield (VA): U.S. Drug Enforcement Administration.

BioSPME, Forensic Toxicology, Fentanyl

K46 Fatal Hydromorphone Overdose in a Child: A Case Report

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The goal of this presentation is to describe the circumstances and postmortem toxicology results in a case of fatal hydromorphone toxicity in a child.

This presentation will impact the forensic science community by contributing to the forensic literature regarding hydromorphone blood concentrations in children. Furthermore, this case provides support for comprehensive drug screening in pediatric deaths.

Hydromorphone is a semi-synthetic opioid analgesic that is approximately eight times more potent when compared to morphine. It is prescribed for the treatment of post-operative or chronic pain; however, it may also be encountered in forensic casework as a drug that is used recreationally. Toxicity due to hydromorphone is dependent on an individual's tolerance, but can include stupor, hypotension, muscle flaccidity, coma, and respiratory depression.

The decedent in this case was a 13-month-old child found vital signs absent in her crib. Emergency medical services were notified, but she was pronounced dead upon arrival at the hospital. Prior to being put to bed the night before her death, she was described as "fussy" and lethargic. The autopsy described a well-developed, well-nourished child, with no significant injuries to head, neck, or torso, and no anatomic cause of death. Heart blood, femoral blood, liver, and stomach contents were submitted for toxicological analysis. Comprehensive drug screening was performed according to a pediatric death protocol used in the province of Ontario, Canada. This protocol comprised: Gas Chromatography/Nitrogen Phosphorous Detection (GC/NPD) and Gas Chromatography/Mass Spectrometry (GC/MS) screen for chemically basic drugs; immunoassay for acetaminophen, salicylates, barbiturates, benzodiazepines, cannabinoid metabolites, cocaine metabolite, opioids (morphine, hydromorphone, codeine, hydrocodone, levorphanol), oxycodone, and fentanyl; Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) analysis for benzodiazepines; and headspace Gas Chromatography/Flame Ionization Detector (GC/FID) for ethanol and other volatiles.

The only toxicologically significant finding in the case was hydromorphone, which was tentatively identified in heart blood by immunoassay and confirmed in femoral blood by a quantitative LC/MS/MS method. The concentration of hydromorphone was determined to be 60ng/mL. Additional case information revealed a variety of prescription and over-the-counter medications used by adults in the home, including hydromorphone in both sustained-release (24mg) and immediate-release (1mg) formulations. The medication history for the deceased included infant acetaminophen drops and a mouthwash preparation comprised of diphenhydramine, aluminum hydroxide and magnesium hydroxide antacid, and lidocaine, prescribed for the treatment of mucositis, mouth pain, and/or oral ulcer.

The scientific literature is of limited assistance with respect to the toxicological interpretation of postmortem blood hydromorphone concentrations in children. Only one previously published case of pediatric overdose provides information on postmortem blood concentrations in children.[1] In that case, postmortem peripheral and heart blood concentrations of 30ng/mL and 60ng/mL, respectively, were measured in a 3-year-old who was determined to have accidentally ingested hydromorphone. Clinical studies are also rare, but provide information on plasma concentrations in children receiving hydromorphone therapeutically. For example, an average plasma concentration of 4.7ng/mL was reported for ten children receiving 2mg hydromorphone intravenously.[2] By comparison, oral administration of a 5mg sustained-release preparation to a 7-year-old child every 12 hours produced a plasma concentration of 1.48ng/mL.[3]

Based on the clinical history, autopsy results, and toxicology findings, the coroner determined the cause of death in this case to be hydromorphone toxicity. The manner of death was undetermined.

Reference(s):

1. Cantrell F.L. et al. A pediatric fatality due to accidental hydromorphone ingestion. *Clin Toxicol.* (Phila). 2017; 55(1): 60-62.

2. Collins J.J. et al. Patient-controlled analgesia for mucositis pain in children: A three-period crossover study comparing morphine and hydromorphone. *Pediatr*. 1996; 129(5): 722-8.

3. Babul N.B., Darke A.C., Hain R.H. Hydromorphone and metabolite pharmacokinetics in children. *J Pain Symptom Manage*. 1995; 10: 335-337.

Hydromorphone, Child, Overdose

K47 An Accidental Death Due to Paraquat Poisoning: An Unusual Case Requiring Toxicologist, Pathologist, and Investigator Collaboration

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After attending this presentation, attendees will better understand the toxicological and pathological findings that indicate death due to paraquat toxicity and how knowledge of the circumstances of death can aid in differentiating between a possible diquat toxicity case and a paraquat toxicity case.

This presentation will impact the forensic science community by demonstrating the necessity of a seamless working relationship between the forensic pathologists, forensic investigators, and toxicologists to understand unusual postmortem cases.

In December 2015, a 38-year-old White female presented to the hospital after accidently drinking from a water bottle that contained weed killer and vomiting twice. The decedent and her husband owned a cleaning business and had obtained the weed killer from the landscaping company at their apartment complex and stored it in a water bottle. The decedent and her husband advised the hospital staff that they believed the liquid to be diquat dibromide and were unsure how much the decedent had consumed before spitting out the liquid.

The hospital staff consulted the Poison Control Center who recommended that the decedent be observed for nine hours before she could be safely discharged. The decedent was treated and discharged later that day. Two days later, the decedent presented to a different hospital after continuing to vomit and complaining of nausea and a burning sensation. Despite medical intervention, her condition deteriorated, and she expired in January 2016, four weeks after the initial ingestion.

Autopsy findings were significant for consolidation of the lungs (right lung weight: 700 grams, left lung weight: 710 grams) with necrosis and purulent exudate. Microscopically, the section of lung exhibited dense pulmonary fibrosis with associated intraparenchymal and intra-alveolar hemorrhages. Delayed pulmonary fibrosis is a characteristic pathological finding in paraquat poisoning that is not seen in diquat poisoning.

A sample of the unknown liquid the decedent drank was submitted to the Miami Dade County Medical Examiner Department (MDME) Toxicology Laboratory for analysis in addition to antemortem samples taken during the decedent's second hospital visit. Toxicological screening of the unknown liquid by Gas Chromatography/Mass Spectrometry (GC/MS) indicated that 4,4-bipyridine was present in the sample. No other analytes were detected in the unknown liquid. 4,4-bipyridine is used as a precursor to paraquat. This finding caused the MDME Toxicology Laboratory to question if the decedent had consumed diquat, as she thought, or if she had consumed paraquat.

Due to the fact that paraquat and diquat could not be distinguished from one another by GC/MS, a fit-for-purpose method was developed by high-performance Liquid Chromatography/Ion Trap/Mass Spectrometry with MS^n capability (LC/Ion Trap/MS^n) to differentiate these two analytes. Analysis of an antemortem urine sample, dated five days after the initial ingestion, by LC/Ion Trap/MS^n indicated that paraquat was present in the sample. The detection of paraquat in a urine sample five days after ingestion is consistent with the literature, which indicates that paraquat can be detected in the urine for up to 26 days after an acute ingestion.

Based on the decedent's history, the sequence of terminal events, autopsy findings, and toxicology findings, the forensic pathologist determined that the cause of death was complications of paraquat toxicity and the manner of death was an accident. This case is a prime example of the necessary working relationship and the ability to share information between the forensic pathologists, forensic investigators, and toxicologists to allow the forensic pathologist to determine a cause of death.

Paraquat, Diquat, Postmortem

K48 Postmortem Tissue Distribution of Synthetic Cathinones

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After attending this presentation, attendees will better understand tissue distributions and the potential for some synthetic cathinones to exhibit significant Postmortem Redistribution (PMR).

This presentation will impact the forensic science community by increasing the fundamental understanding of synthetic cathinone distribution in postmortem toxicological samples and the influence of PMR.

Postmortem toxicology results can provide crucial information in death investigations regarding the cause and manner of death; however, postmortem drug concentrations may not always reflect antemortem concentrations. Drugs may also undergo PMR, resulting in significant differences between central and peripheral blood. To assess PMR, Central to Peripheral (C/P) blood ratios can be calculated. PMR can be somewhat predicted using drug properties, including volume of distribution, lipophilicity, and p*Ka*. Blood and tissue distributions have been studied for many common illicit drugs, but this information is still limited for synthetic cathinones, a class of designer drugs that has been increasing in popularity over the past decade. In this presentation, postmortem tissue distributions and C/P ratios for select synthetic cathinones from cathinone-positive fatalities will be presented.

Postmortem samples from 60 cathinone-positive cases were included in the study. A total of 210 specimens were evaluated, including liver, urine, vitreous humor, and blood collected from the aorta, inferior vena cava, iliac, subclavian, and femoral vessels. Quantitative analysis was performed using blood or urine calibrators with matrix-matched controls. Samples were analyzed using a previously published and validated procedure for the determination of 22 synthetic cathinones in urine and blood using Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS). A total of nine isotopically labelled internal standards were used. The principal compounds of interest were methcathinone, 3-fFuoromethcathinone (3-FMC), 4-Fluoromethcathinone (4-FMC), ethcathinone, ethylone, methedrone, buphedrone, butylone, mephedrone, eutylone, 4-Methylethcathinone (4-MEC), 3,4-Methylenedioxy-α-Pyrrolidinobutyrophenone (MDPBP), pentedrone, pentylone, 3,4-Dimethylmethcathinone (3,4-DMMC), α-Pyrrolidinopentiophenone (α-PVP), 4-Ethylmethcathinone (4-EMC), 4-Methyl-α-Pyrrolidinobutiophenone (MPBP), Methylenedioxypyrovalerone (MDPV), pyrovalerone, and naphyrone.

Of the 22 cathinones in the assay, 9 were identified in at least one case: α-PVP (*n*=18), methylone (*n*=17), ethylone (*n*=15), MDPV (*n*=6), pentylone (*n*=3), methedrone (*n*=2), 4-MEC (*n*=1), butylone (*n*=1), and MDPBP (*n*=1). Concentration ranges in blood, urine, and liver, respectively, were <2 to 1,090ng/mL, 33 to 7,580ng/mL, and 14 to 663ng/g for α-PVP; <2 to 202 ng/mL, 2 to 38,064ng/mL, and 28 to 5,731ng/g for methylone; <2 to 2,743ng/mL, 32 to >20,000ng/mL, and 10 to 18,893ng/g for ethylone; 3 to 80ng/mL, 4 to 5,210ng/mL, and 64 to 840ng/g for MDPV; <5 to 322ng/mL in blood and 122 to >5,000 in urine for pentylone. Where possible, average C/P ratios were determined as follows: methylone (4.0, range 2.39-6.0, *n*=4), ethylone (2.9, range 0.5-9.2, *n*=6), pentylone (2.0, *n*=1), α-PVP (1.2, range 0.5-1.9, *n*=8), methedrone (1.1, *n*=1), MDPV (1.0, *n*=1), and butylone (0.7, *n*=1). Although C/P ratios were highly variable, some cathinones appeared to have significant potential for redistribution. Generally, the highest C/P ratios were observed in methyenedioxy-type cathinones bearing secondary amines. Although the pyrrolidine-type cathinones are less polar and subsequently more lipophilic, they are less basic than their secondary amine counterparts. Vitreous humor was only available in a small number of cases, but concentrations in vitreous were comparable to peripheral blood within this limited population. Although the concentration range of forensic interest was wide, the results also highlight the need for low limits of detection and quantification.

Tissue distributions and C/P ratios are presented and compared with existing literature. Variability of C/P ratios and the potential for cathinones to degrade *in situ* and during storage significantly complicates their interpretation. Of the 60 cases submitted, 50 cases had at least one specimen test positive for a synthetic cathinone. The results highlight the potential for some cathinones to exhibit PMR, the importance of collecting multiple specimens, and the interpretation of results within the full context of investigative information.

Synthetic Cathinones, Postmortem Redistribution, LC/qTOF/MS

K49 Acute Intoxications With Phenibut (ß-Phenyl- γ -Aminobutyric Acid), an Emergent Psychoactive γ -Aminobutyric Acid (GABA) Agonist

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After attending this presentation, attendees will be familiar with the behavioral and physiological effects of the psychoactive compound known as phenibut. Attendees will also understand the importance of updated screening libraries for the detection of Novel Psychoactive Substances (NPS).

This presentation will impact the forensic science community by raising awareness regarding phenibut abuse and by providing examples of screening and confirmation techniques for this analytically challenging substance.

Phenibut is an analogue of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and the antispasmodic baclofen. Neuromodulation occurs when phenibut binds to GABA receptors, primarily at $GABA_B$. While not licensed for medical use in the United States, phenibut has been prescribed in Russia since the 1960s for the treatment of anxiety, alcohol withdrawal, and insomnia. It is currently available on the internet as a nutritional supplement and marketed as a nootropic, anxiolytic, and euphoriant, where typical doses range from 500mg to 2g. Phenibut tolerance develops rapidly, similar to other GABA receptor modulators. Tolerance may precipitate substantial dose increases. Additionally, co-administration with other GABA modulators, such as ethanol, may result in more pronounced pharmacological effects. In this submission, two unrelated cases involving phenibut intoxication are presented.

Case 1: A 21-year-old male was found unconscious in a dormitory hallway. The subject was minimally responsive, slurring his words, and walking into walls when emergency personnel arrived. The man vomited, his condition deteriorated, and he was transported to the hospital. The attending physician described his aggressive and combative behavior as excited delirium. He assaulted medical staff, was restrained, and remained in the hospital for two days.

Case 2: A 21-year-old male was unconscious and unresponsive in a dormitory stairwell the morning after a night of drinking with friends. Two individuals were sent to retrieve the man and found him staggering and slurring his speech. The man was taken to his dorm room, where he laid down and fell asleep. His roommate attempted to wake him 1h later, but he appeared incoherent and confused. Medical personnel applied multiple sternum rubs to revive the individual. He jolted awake, appeared disoriented, and his pupils were non-reactive to light stimulus. He was transported to the hospital where toxicology revealed no drugs or alcohol.

Urine from each case was submitted to the Armed Forces Medical Examiner System (AFMES) Division of Forensic Toxicology. Both specimens were negative for ethanol by gas chromatography/flame ionization detection and for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, opioids, phencyclidine, and sympathomimetic amines by immunoassay. In addition, both cases screened negative for alkaline-extractable drugs by gas chromatography/full-scan mass spectrometry. After case histories were reviewed, a non-targeted liquid chromatography/quadrupole time of flight/mass spectrometry drug screen was added. In Case 1, phenibut and ondansetron were detected in the urine. Phenibut, naloxone, and ondansetron were detected in the urine from Case 2. Phenibut was confirmed in the urine from both cases by liquid chromatography/tandem mass spectrometry.

Unknown to the lab at time of analysis, the individual in Case 1 later admitted to self-medicating with an internet-purchased supplement for his social anxiety and attention-deficit hyperactivity disorder. On the date of the incident, he self-reported ingesting 20g of phenibut, causing him to vomit shortly thereafter. He then ingested an additional 10g in an attempt to account for the amount he regurgitated. The individual in Case 2 was interviewed after discharge and stated he drank wine, beer, and champagne the night prior to the incident. He did not recall any events 8h prior to medical intervention in his dormitory room. The individual also admitted to purchasing phenibut from the internet, but did not provide information concerning the amount ingested or if the dose was co-ingested with alcohol.

In summary, two acute intoxications with phenibut that emphasize the difficulties encountered during extraction and instrumental analysis are presented. If proper analytical techniques are not available, methods are not current, or history is incomplete, it is possible to overlook phenibut and other NPS. In these cases, history was a key factor in directing additional testing. Intoxication effects corroborated previous reports of somnolence/stupor, confusion, agitation, nausea, and vomiting. These cases underscore the need for vigilance when evaluating casework and promote the use of comprehensive screening techniques that may reveal rare, but significant, findings.

Phenibut, NPS, QTOF

K50 A Forensic Characterization of Bacterial and Fungal Organisms in Traditional Chinese Herbs

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After attending this presentation, attendees will be aware of the potential presence of toxic microorganisms in Chinese herbal products used for medicinal purposes.

This presentation will impact the forensic science community by potentially being used to identify unknown specimens of herbs, provide provenance of the herbs themselves, and help reconstruct toxicological episodes that result in medical emergencies or death.

Traditional Chinese Medicine (TCM) is one of the oldest healing methods used in Chinese culture, often referred to as "formula." TCM's herbal formulations are prescribed for a variety of illnesses, based on symptoms presented. Herbal remedies are selected based on Chinese ancient literature and individual experiences of patients or doctors. Use of TCM in the United States has increased because herbal remedies are believed to be less expensive and more effective with less adverse effects in comparison to traditional pharmaceutics. Therefore, sales have increased, despite articles and case studies that have demonstrated the dangers, such as injury and death, related to TCM, stemming from improper labelling, toxic contaminants, and, in some cases, the presence of pathogenic bacteria.

Objective: The purpose of this study was to conduct a molecular and biochemical survey of microorganisms of 11 Chinese herbal products purchased from a traditional medicine shop in Beijing, China. Bulk analysis of microbial/fungal lipids from the herbs was conducted using a rapid method for extraction of cellular fatty acids and derivatization into Fatty Acid Methyl Esters (FAMEs) prior to profiling with Gas Chromatography/Flame Ionization Detector (GC/FID).

Methods: Eleven over-the-counter Chinese herbs were purchased from Tong Ren Tang in Beijing, China. These herbs were chosen based on reported pharmacological activity: sedative and hypnotic (Suan Zao Ren, Fu Ling, Sha Yuan Zi, and Di Long); anticonvulsant (Gou Teng, Tian Ma, and Jiang Can); and analgesic (Yan Hu Suo, Chuan Duan, Wu Yao, and Mo Yao).

Approximately 100mg of the herbal product was placed in 1x Phosphate Buffered Saline (PBS). A 100µL aliquot of the solution was spread onto Tryptic Soy Agar (TSA) with and without blood supplements (50mg/L). Plates were then incubated overnight at 30°C. Colony growths were photographed, harvested, and subjected to FAME profiling.

FAME profiling of the herbal products was performed using GC/FID equipped with a series of analytical standards to detect and quantify fatty acids between 9 and 20 carbons in length.

Products were incubated with methanolic potassium hydroxide (5% KOH, 95% CH_3OH). The methyl esters were then extracted into hexane and analyzed. The individual fatty acids were identified by their retention time through comparison to reference standards.

Results: Strains within the *Bacillus* group were identified in nearly all 11 of the herbal samples. These included *B. subtilis* and *B. cereus*, as well as *B. megaterium*, *B. circulans*, and *B. atrophaeus*. Organisms belonging to the *Bacillus* ACT group (anthracis, cereus, thuringiensis) were identified in 5 out of 1 herb cultures as evidenced by the large ratio of 15:0 iso to 15:0 anteiso fatty acid biomarkers. A gram-positive, aerobic bacteria related to the *Bacillus* group, *Paenibacillus thiaminolyticus*, was also detected. This bacteria differs from *Bacillus* ACT and has been reported to cause bacteremic infections in humans. All of the herbal specimens also exhibited fungal biomarkers such as polyunsaturated 20:4 ω6,9,12,15c, and 18:3 ω6c (6,9,12). The presence of fungal biomarkers would be consistent with the origin of some herbal samples such as Jiang Can, silkworm larvae that are claimed to have been killed with the fungus *Beauveria bassiana*; however, in others, they could represent contamination of fungal spores.

Conclusion: The characterization of microorganisms present in these traditional Chinese herbs was successful through analysis of their FAME profiles and by the presence of particular and unique fatty acids. The bacterial and fungal identification can potentially be used to identify unknown specimens, provide provenance of the herbs themselves, and help to reconstruct toxicological episodes that result in medical emergencies or death.

Chinese Herbs, FAME Analysis, GC/FID

K51 The Role of Toxicology in Child Custody Disputes

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After attending this presentation, attendees will be able to discuss the importance of good laboratory practices, the pharmacology of drugs in analysis, and the interpretation of specimens in child custody cases.

This presentation will impact the forensic science community by describing the role of toxicology in child custody disputes.

Two cases are presented which highlight the importance of validated testing and interpretation of results in child custody cases. For both cases, a private toxicology lab (Lab A) was contacted and asked to review the analytical work performed at other laboratories, review the reported findings, and/or comment on opinions provided by other scientists.

Case 1: The mother of three children was accused of exposing them to Gamma Hydroxybutyrate (GHB), diazepam, and cocaine. Lab A performed hair testing; Lab B performed blood and segmented hair analysis on the mother and all the children; and Lab C tested urine, hair, and blood form both parents and the children. In total, more than 50 different tests were performed at Lab C. The following results will be discussed: diazepam in blood and urine specimens from both adults; diazepam in hair of all tested individuals at concentrations of 39.3ng/mg–215ng/mg; GHB in hair of all tested individuals at concentrations of 76.3ng/mg–225.5ng/mg; and methylecgonine in one hair segment from child 1 at a concentration of 39.3ng/mg. Despite testing hair specimens covering the same time range with reporting limits well below the results reported by Lab C, Labs A and B had no positive findings.

Despite the fact that analytical data was not available for review, the following inconsistencies between the known disposition of analytes in biological matrices and previously reported findings were identified: diazpeam was reported in blood and urine specimens in the absence of nordiazepam or temazepam though the reports indicated these analytes were in the scope of testing; diazepam concentrations in hair were approximately 1,000 to 10,000 times higher than the concentrations reported in women with known dosing regimens of the drug and at least five times higher than those reported in a drug abuser; nordiazepam was reported in the hair despite published studies that indicate that nordiazepam concentration typically exceeds those of diazepam in this matrix; GHB concentrations reported in this case were 76ng/mg–225ng/mg while one reported case of an individual given multiple doses had a maximum hair concentration of 1.66ng/mg; and, methylecgonine levels reported in the hair segments were at least 25x higher than what has been reported in patients who were administered cocaine, and neither cocaine nor benzoylecgonine were detected.

Case 2: A father was accused of exposing two children to phensuximide. Lab D performed testing on a powder found in the home and reported no drugs found. The data was forwarded to a chemical engineer (Dr. X, who concluded that the powder was 88% phensuximide. Subsequently, urine specimens were collected from the children and analyzed by the chemical engineer at Lab E, who indicated in a deposition that the urine samples contained succinimide and phenol, which he concluded were metabolites of phensuximide, proving exposure.

The data from Lab D and multiple reports, letters, and the deposition of Dr. X were provided to Lab A for review; no data was made available from Lab E. Based on the review of the available data and the deposition of Dr. X, it appeared as if Dr. X concluded that a large peak in Lab D's data was phensuximide based on the results of Lab D's in-house library search, which identified the peak as phensuximide with a match factor of 50; however, comparison of the spectrum of the unknown to the spectrum of phensuximide proved that this conclusion was not valid. Additionally, there is no literature or metabolic pathways that support the conclusion that succinimide or phenol are metabolites of phensuximide. A review of the data and comparison to known mass spectra led to the preliminary conclusion that the large unknown peak from the brown powder that smelled like cinnamon was cinnamaldehyde and none of the testing performed provided any evidence of phensuximide exposure.

Child Custody, Hair Testing, Jurisprudence

K52 A Segmental Analysis of Endogenous Gamma-Hydroxybutyric (GHB) Acid in Human Hair

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After attending this presentation, attendees will be informed about a validated Liquid-Liquid Extraction (LLE) and Liquid Chromatograph/Tandem Mass Spectrometric (LC/MS/MS) method for the detection of GHB in human head hair. The validated method uses multi-point calibration curves and quality control samples. The research presented also investigates segmental analysis of hair samples and inter- and intra-variation among individuals.

This presentation will impact the forensic science community by providing information regarding endogenous GHB concentrations in hair. The research presented will address baseline levels of GHB within and between individuals to determine if a background threshold can be established for an unexposed population. Segmental analysis of hair samples will provide information regarding the variation of endogenous concentrations within an individual over a period of time that corresponds to hair growth rates.

GHB has been used in drug-facilitated crimes and is also a popular recreational drug of abuse. Ingestion of this drug may induce euphoria, amnesia, dizziness, and unconsciousness, depending on dosage. Because GHB is a natural chemical found in humans, it can be difficult to separate naturally occurring levels from levels following ingestion. GHB poses an additional challenge to the forensic community in that it is rapidly excreted by the body. Hair analysis is a good alternative to blood and urine due to the longer detection window available to establish the involvement of drugs in reported crimes. The scientifically accepted mean growth rate of human head hair is 1cm per month, which can be used to estimate the time period of ingestion. This research utilizes segmental hair analysis to determine baseline GHB concentrations among non-GHB users and to evaluate variability along the length of the hair. Knowing how much variation is present within an individual will help determine if an individual can serve as their own control in cases of ingestion.

Before collecting hair samples, a full quantitative validation was performed for the extraction procedure. The parameters assessed were: accuracy, precision, calibration model, carryover, interferences, Limit Of Detection (LOD), Limit Of Quantitation (LOQ), and processed sample stability. Due to the endogenous nature of GHB in hair, ionization suppression/enhancement experiments were not completed. Instead, the study relied on the deuterated GHB to compensate for any suppression or enhancement that may occur. Accuracy and precision were found to be within ±20% at low (1.2ng/mg), medium (4.0ng/mg), and high (9.6ng/mg) concentrations. A linear model was obtained from 0.4ng/mg to 12ng/mg and no carryover was observed in unspiked synthetic hair samples following injections of 12ng/mg or 24ng/mg GHB. No interfering signals (not including background GHB) were observed in hair extracts. The LOD and LOQ of the method were experimentally determined to be 0.4ng/mg. Lastly, extracts were observed to be stable after eight days while being stored at ≤14°C.

To evaluate the baseline GHB concentrations, hair collected from non-GHB users was segmented into 1cm increments based on proximity to the scalp. The segments were washed with organic solvents, cryogenically ground, and digested with sodium hydroxide. After digestion, the samples were neutralized with sulfuric acid and extracted via LLE with ethyl acetate. The extracts were then evaporated to dryness, reconstituted in mobile phase, and filtered for analysis by LC/MS/MS to determine the levels of GHB present. Initial results for 37 non-GHB users reveal an average baseline GHB concentration of 0.90ng/mg, with a minimum of 0.43ng/mg, maximum of 3.49ng/mg, and median of 0.84ng/mg. The average variation within individuals was 13%. Work continues on the processing of additional hair samples from other non-drug users.

GHB, Hair Analysis, LLE

K53 A Wastewater Analysis for Tobacco and Drug Detection in New York City

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After attending this presentation, attendees will understand the utility of wastewater analysis to monitor tobacco and drug exposure in a certain community and will know how to perform the analysis of these types of samples by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).

This presentation will impact the forensic science community by demonstrating the applicability of utilizing wastewater analysis by LC/MS/MS to investigate tobacco and drug use in different communities in an urban location.

Recent changes in the drug scene, such as the current issues with prescription opiates and fentanyl, among other substances, have sparked an increased interest in new tools to monitor what drugs are coming onto the market in a faster, more efficient manner than conventional population surveys. Wastewater analysis is an innovative approach to testing the drug consumption in a geographical area by analyzing human excretion products (biomarkers) in wastewater, which is essentially a large urine pool. Wastewater can provide independent, low-cost, reliable, and nearly real-time information. This methodology has not been fully explored in the United States.

A method was developed to determine tobacco (nicotine and cotinine), cocaine (benzoylecognine, cocaethylene, and cocaine), amphetamines (methamphetamine, MDMA, MDA, and amphetamine), opiates (6-monoacetilmorphine, morphine, codeine, oxymorphone, oxycodone, hydromorphone, hydrocodone, fentanyl, norfentanyl, methadone, EDDP), and cannabis (delta-9-tetrahydrocannabinol, 11-nor-9-carboxy-tetrahydrocannabinol, and 11-nor-9-carboxy-tetrahydrocannabinol-glucuronide) biomarkers in 50mL of wastewater. Wastewater samples were filtered, extracted using mixed-mode cation cartridges, and analyzed by LC/MS/MS using positive Electrospray Ionization (ESI). All compounds were analyzed on a Kinetex® C18 column (with 0.1% formic acid in water and 0.1% formic acid in acetonitrile as mobile phases) using two different gradients (one for cannabinoids and another for the remaining compounds). Each compound was monitored by two Multiple Reaction Monitoring (MRM) transitions. Method validation included linearity (5ng/L-1,000ng/L for all compounds, except 10-1,000ng/L for tobacco biomarkers), limit of detection (1ng/L-10ng/L) and quantification (5ng/L-10ng/L), imprecision (<20%), accuracy (80%-120%), matrix effect and extraction efficiency, interferences, and auto-sampler stability. This study applied this method to wastewater samples collected from wastewater treatment plants in New York City (The Bronx, Brooklyn, Queens, and Manhattan) throughout one year. Wastewater samples were collected into Environmental Protection Agency (EPA) -certified sample containers and stored at −20°C until analysis.

This study emphasizes the method of analysis, particularly the use of LC/MS/MS in terms of its sensitivity and selectivity, as a means to detect licit and illicit drugs in wastewater samples. It also provides a means by which new drug trends could be tracked by testing wastewater, thus providing real-time results in different boroughs within New York City.

Wastewater, Cannabis, Opiates

K54 Postmortem Pediatric Toxicology

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After attending this presentation, attendees will gain an appreciation for the challenges unique to toxicological findings in postmortem pediatric cases. Attendees will learn interpretive guidelines for pediatric cases involving forensic toxicology in both a general and case-specific sense.

This presentation will impact the forensic science community by further delineating the interpretive aspects of toxicological findings in the pediatric population.

In this 19th Annual Special Session within the Toxicology section, pediatric cases involving toxicological findings will be discussed. As a relative dearth of interpretive information exists involving toxicological findings in the pediatric population, this session is a forum to help elucidate and clarify such issues. The format is a short case presentation or issue-specific concern, including pharmaco-toxicokinetic data and other relevant ancillary information, followed by audience participation to provide interpretive clarity around case-specific impacts of the toxicological findings. This session, attended by various sections of the Academy, allows for various perspectives of case issues that lead to integrative consensus, or differing opinions, as to cause of death in children.

Four cases will be presented that highlight the difficulty in assessing the role of toxicants in each case or the lengths to which one must go, in some cases. Richard Harruff, MD, PhD, Carl Schmidt, MD, Thomas Rosano, PhD, and Robert Middleberg, PhD, will be reviewing cases from their years of experience as forensic pathologists and toxicologists, respectively, that highlight the issues and confounders in the pediatric population.

Dr. Harruff will discuss a case involving colchicine. This esoteric substance in relation to the pediatric population has a number of therapeutic uses based on being an inhibitor of mitosis, including one of the primary treatments for gouty arthritis. The case presentation will address potential means of exposure, post-exposure effects, and consequences of colchicine exposure in the pediatric population.

Dr. Schmidt will discuss the case of twins who were administered lidocaine-containing teething gel. The toxicokinetics and toxicodynamics of this commonly used local anesthetic and cardio-active substance will be reviewed in relation to adverse outcomes, especially in the pediatric population. The risks associated with use of teething gels, in particular, will be emphasized.

Dr. Rosano will present a case involving the death of an 18-month-old who came in contact with liquid nicotine intended to be used in electronic cigarettes. This case will highlight how the child came in contact with the material, surrounding factors leading to exposure, the signs and symptoms after exposure, and postmortem toxicological findings. Finally, the outcome of the investigations will be addressed.

Dr. Middleberg will discuss the difficulties associated with assigning manner of death in pediatric cases. Special emphasis will be placed on the issues surrounding child endangerment versus homicide and the difficulties associated with the distinction. An open discussion regarding whether there is ever a way to readily move from child endangerment to homicide in toxicologically related cases will take place.

Pediatric, Postmortem, Toxicology