2020 ONLINE FORENSIC SYMPOSIUM
Final Program & Abstracts

June 8th - 12th, 2020
Current Trends in Forensic Toxicology
FREE Registration / CE Credits Available

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Welcome to the 3rd Annual Online Forensic Symposium. It is my great pleasure to welcome you to the first event in this year’s “Online Forensic Symposium – Current Trends in Forensic Toxicology.” The demand for information, continuing education and international cooperation has continued to increase, fueling the growth of this Symposium from a 3-day event in 2018 to a series of week-long events covering forensic toxicology, seized drugs, and trace analysis.

As the event has expanded, I have required more assistance in ensuring the exceptional quality of technical content that you have come to expect from the Symposium. Consequently, I am thrilled to have two outstanding Program Chairs – Prof. Simon Elliott & Prof. Nikolas Lemos, who have voluntarily dedicated their time and talents in developing this year’s program. I can’t thank them enough for all that they have done. Similarly, my heartfelt thanks go out to all of this year’s speakers who are volunteering their time and sharing their knowledge and expertise for the benefit of the broader community.

Another change this year is the Symposium’s new home: The Center for Forensic Science, Research & Education (CFSRE). I am deeply grateful to the CFSRE team, who has worked tirelessly to create a special place for the Symposium to reside. The alignment of our mission and vision with regard to education, professional development and international outreach in the areas of Forensic Chemistry/Toxicology and Forensic Biology has resulted in a wonderfully synergistic partnership.

Finally, I want to thank all of this year’s sponsors for allowing this week to happen. As you know, there is no registration fee – the entire event is free of charge to anyone who wishes to attend. For those unable to join live, the Symposium will be recorded and made available in an on-demand format. Despite the many volunteer hours that go into producing this event by myself, the speakers, the Program Chairs, and the CFSRE, there are still costs that need to be covered. If it were not for the generosity of our friends at Agilent Technologies, GERSTEL, Shimadzu, Wiley and NMS Labs, this event would not be possible. Please do take the time to listen to their presentations, honestly complete the registration questionnaire which provides them with valuable feedback and support these businesses whenever possible just as they are supporting you this week.

Thank you, and I hope you enjoy this opportunity to Learn without Leaving the Lab!

Warm Regards,

Tom Gluodenis, MBA, PMFS, Ph.D.,
Assistant Professor, Lincoln University, PA
Organizer & Vendor Liaison
Online Forensic Symposium: Forensic Toxicology
info@ForensicSymposium.org
linkedin.com/in/tgluodenis
“Current challenges in forensic toxicology...and future solutions?”

Despite many innovations and increased knowledge within forensic toxicology, there are many challenges that persist. This could be accepted if they were not too important, however these challenges are in those areas still fundamental to the role and application of forensic toxicology such as analysis, interpretation (especially in fatalities) and court testimony. Advancements in analytical toxicology have enabled the detection of more substances with increasing sensitivity and in a variety of matrices, exemplified by the increasing use of accurate mass-spectrometry. Furthermore, on-going developments in physics and chemistry have already enhanced detection systems in other fields but could soon be applied to toxicology. Nevertheless, even with greater detection and accuracy, it is necessary to interpret analytical findings and as more and more factors are identified that can influence that interpretation, this results in more aspects to understand and consider. Indeed toxicology is a discipline whereby the more you know, the less you realise you know. However, researchers are making significant progress in recognising these factors as well as greater understanding of old ones and determining approaches and considerations that can be applied to aid interpretation of toxicology results. Even then, it is important to be able to convey results and interpretation appropriately in a variety of formats and settings, not least within Courts, which is a primary challenge faced by toxicologists around the world despite working within different legal systems. In fact in recent years all of these analytical, interpretative and testimony challenges have applied to cannabis and cannabinoids, including synthetic cannabinoids. The intention of this forensic toxicology week is to highlight these current challenges for awareness and increase knowledge and maybe show a glimpse of the future where we are finding solutions. Enjoy the week.

Prof Simon Elliott, PhD – Chair
Prof Nikolas Lemos, PhD – Co-Chair
Prof. Simon Elliott
Scientific Program Chair
Director, Elliott Forensic Consulting, Ltd.

Prof Simon Elliott has over 20 years’ experience in forensic toxicology and is a Consultant Forensic Toxicologist, independent Business Consultant and Director of Elliott Forensic Consulting Ltd. He was previously the Director of Global Forensics at Alere Inc (now part of Abbott) (2017-2018) having also been the founder and Managing Director of Forensics Ltd (ROAR Forensics, subsequently Alere Forensics) in Malvern, Worcestershire, UK (2008-2017). Prof Elliott previously worked as a Clinical Scientist in the NHS at Birmingham City Hospital for over 10 years specifically involved in clinical and forensic toxicology as Section Head of Forensic Toxicology. He is a Visiting Professor in Forensic Toxicology at King’s College London and holds a BSc in Biochemistry from the University of Bath and a PhD in Biochemical Toxicology (studying GHB) from the University of Birmingham. He is a Chartered Scientist and European Registered Toxicologist as well as being a member of a number of professional organisations including The International Association of Forensic Toxicologists (TIAFT, Board Member), the LTG, Chartered Society for Forensic Science, Association of Clinical Biochemistry and Royal Society of Chemistry in addition to being a founder and Chair of the UK & Ireland Association of Forensic Toxicologists (UKIAFT). An author of over 70 scientific publications, articles and book chapters, he is on the Editorial Board of Drug Testing & Analysis and Forensic Science International journals as well as being an Associate Editor of the Journal of Analytical Toxicology and an Editor of Wiley’s WIREs Forensic Science (Toxicology) and Clarke’s Analysis of Drugs and Poisons. Prof Elliott has presented at many national and international meetings (including invited speaker) as well as presenting expert evidence for many years in Coronal, Civil and Criminal Court. A member of the World Health Organisation (WHO) Expert Committee on Drug Dependence, he also advises the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) and United Nations Office on Drugs and Crime (UNODC), especially regarding new psychoactive substances.
Dr. Lemos is a board-certified forensic toxicologist, academic and researcher who started his career researching alcohol and drug detection analysis in alternative biological specimens. After getting awarded his doctorate in Forensic Medicine and Science (Analytical and Forensic Toxicology) from the University of Glasgow in Scotland, he worked as Senior Lecturer at London South Bank University where he helped develop a new undergraduate course in forensic science. He later moved to St George’s Hospital Medical School of the University of London to assume the position of Head of the Forensic Toxicology Service of its Analytical Unit where he supervised or co-supervised the research of numerous medical and graduate students who trained in the Analytical Unit.

In 2003, he became Chief Forensic Toxicologist and in 2005 Director of the Forensic Laboratory Division at the Office of the Chief Medical Examiner of the City and County of San Francisco where he spearheaded that office’s ABFT accreditation. He is actively engaged in various international professional organizations and is a Member of the Chartered Society of Forensic Scientists, Fellow of the Royal Society of Chemistry, Fellow of the American Board of Forensic Toxicology, and Fellow of the American Academy of Forensic Sciences. Dr. Lemos has consulted and testified in over 335 civil and criminal trials around the world. He has co-authored over 85 peer-reviewed papers, abstracts, and book chapters including the first ever study of Cannabinoids in Fingernails (JAT, May 1999) and the first ever study of Cannabinoids in Post-mortem Toxicology (JAT, September 2011). He has organized and hosted numerous professional scientific meetings in the USA and abroad including the 2011 Joint SOFT-TIAFT Meeting held in San Francisco with over 1,500 participants from 60 countries in attendance. He has previously served as Science Editor of “Medicine, Science and the Law,” the Official Journal of the British Academy of Forensic Sciences, and he is the holder of many scholarships, research and equipment grants and awards. In 2005, he was honoured with a Proclamation of Achievement by the 109th Congress of the United States of America “for dedicating his life and career to community safety and social awareness.”

especially regarding new psychoactive substances.
Dr. Tom Gluodenis
Symposium Organizer
Assistant Professor, Lincoln University, PA
linkedin.com/in/tgluodenis

Dr. Tom Gluodenis earned a PMFS from Florida International University, an EMBA from St. Joseph University in Philadelphia and his MSc. and Ph.D. in analytical chemistry from the University of Massachusetts, Amherst. He spent 23 years with Hewlett-Packard/Agilent Technologies most recently as Director of Homeland Security Programs and the Global Business Manager for Forensics & Forensic Toxicology. In those roles, Dr. Gluodenis was an expert resource on forensic trends, regulations, technologies, and testing protocols while coordinating countless partnerships & collaborations with practitioners and researchers around the globe. In addition to organizing the annual Online Forensic Symposium Series and his appointment as an Assistant Professor at Lincoln University in Pennsylvania, Dr. Gluodenis currently serves on several consensus bodies including ASTM E30, the American Standards Board, and the NIST Organization of Scientific Area Committees. He is also a member of a number of national and international forensic organizations including the Society of Forensic Toxicologists, the American Academy of Forensic Sciences, the International Association of Forensic Toxicologists, and the Forensic & Clinical Toxicology Association of Australasia.
The mission statement of the CFSRE is to advance the field of forensic science for future and current practitioners as well as members of the justice system by providing innovation, mentorship, advanced technology and expertise to promote progress and quality in the forensic sciences. For more information on our continuing education and professional development programs, please visit: www.CFSRE.org
Monday – June 8th, 2020

Post-Mortem Changes

9am EST – 9:15am EST
1pm GMT – 1:15pm GMT

Welcome & Introduction
Prof. Simon Elliott & Prof. Nikolas Lemos,
Scientific Program Chairs

9:15am EST – 10:15am EST
1:15pm GMT – 2:15pm GMT

Cadaverous Changes & Post-Mortem Toxicology
Federica Bortolotti, MD, PhD, Associate Professor of Legal Medicine,
Unit of Forensic Medicine, Dept. of Diagnostics and Public Health,
University of Verona, Verona, Italy

10:15am EST – 10:30am EST
2:15pm GMT – 2:30pm GMT

Kratom and Mitragynine in Forensic Casework (sponsored)
Dr. Barry K. Logan, PhD, D-ABFT
Chief Scientist, NMS Labs

10:30am EST – 11:30am EST
2:30pm GMT – 3:30pm GMT

Studies on (Time-Dependent) Postmortem Redistribution – Application of CT-Guide Biopsy Sampling
Dr. Andrea Steuer, Department of Forensic Pharmacology and Toxicology,
Zurich Institute of Forensic Medicine, University of Zurich

11:30am EST – 12:00pm EST
3:30pm GMT – 4:00pm GMT

Panel Discussion
All of the day’s speakers
Tuesday – June 9th, 2020

Inter Individual Variations & Toxicology Interpretation

9am EST – 10:00am EST
1pm GMT – 2:00pm GMT

Reference Postmortem Blood Drug Concentrations
Professor Henrik Druid, Karolinska Institutet, Stockholm, Sweden

10:00am EST – 10:15am EST
2:00pm GMT – 2:15pm GMT

High Resolution Screening of NPS in Postmortem Samples (sponsored)
Sarah Olive, Mass Spec Technical Support Scientist, Shimadzu Scientific Instruments

10:15am EST – 11:15am EST
2:15pm GMT – 3:15pm GMT

Pharmacogenomic Testing for Forensic Toxicology
Alan WU, Ph.D., University of California, San Francisco (UCSF)

11:15am EST – 11:30am EST
3:15pm GMT – 3:30pm GMT

Ultrafast Screening of 263 Drug Targets in Blood (sponsored)
Kevin McCann, Product Owner for RapidFire and StreamSelect, Agilent Technologies

11:30am EST – 12:00pm EST
3:30pm GMT – 4:00pm GMT

Panel Discussion
All of the day’s speakers
Wednesday – June 10th, 2020
Innovations in Analysis

9am EST – 10:00am EST
1pm GMT – 2:00pm GMT

**Molecularly Imprinted Polymers for the Detection of Drugs of Abuse**
Miss Fabiana Grillo, University of Leicester, Leicester, LE1 7RH, United Kingdom

10:00am EST – 10:15am EST
2:00pm GMT – 2:15pm GMT

**Automated Determination of Phosphatidylethanol (PEth) from Dried Blood Spots (DBS)(sponsored)**
Dr. Oliver Lerch, GERSTEL GmbH & Co. KG, Muelheim, Germany

10:15am EST – 11:15am EST
2:15pm GMT – 3:15pm GMT

**High Resolution LC-MS in Forensic Toxicology – Trials and Tribulations, Tips and Tricks**
Dr Lewis Couchman, Analytical Services International, London, U.K.

11:15am EST – 11:30am EST
3:15pm GMT – 3:30pm GMT

**Routine Screening of Drugs in Whole Blood Using LC/Q-TOF (sponsored)**
Dr. Karen E. Yannell, LC/MS Application Scientist Agilent Technologies, Santa Clara, CA

11:30am EST – 12:00pm EST
3:30pm GMT – 4:00pm GMT

**Panel Discussion**
All of the day’s speakers
Thursday – June 11th, 2020

Challenges of Cannabis & Cannabinoids

9am EST – 10:00am EST
1pm GMT – 2:00pm GMT

Cannabis And Cannabis Derived Products: Hot Topics Likely to Influence Your Practice As A Forensic Scientist.
H. Chip Walls, Forensic Analytical & Clinical Toxicology Consultant and Training Specialists, Miami, FL, USA

10:00am EST – 10:15am EST
2:00pm GMT – 2:15pm GMT

Discovery of NPS in Forensic Toxicology Casework (sponsored)
Dr. Alex J. Krotulski, Research Scientist, Center for Forensic Science Research and Education (CFSRE), Willow Grove, PA USA

10:15am EST – 11:15am EST
2:15pm GMT – 3:15pm GMT

Pharmacology and Toxicology of Synthetic Cannabinoids
Prof. Dr. Volker Auwärter, Forensic Toxicology, Institute of Forensic Medicine, Medical Center – University of Freiburg, Freiburg, Germany

11:15am EST – 11:30am EST
3:15pm GMT – 3:30pm GMT

Eliminating Backlog for Forensic Analysis with Intuvo 9000 GC Technology (sponsored)
Dr. Rebecca Veeneman, Applications Chemist Manager, Agilent Technologies, Wilmington, DE

11:30am EST – 12:00pm EST
3:30pm GMT – 4:00pm GMT

Panel Discussion
All of the day’s speakers
Friday – June 12th, 2020

Court Testimony

9am EST – 10:00am EST
1pm GMT – 2:00pm GMT

Title TBA
Dr. Andrew Mubiru, Uganda Police Force

10:00am EST – 10:15am EST
2:00pm GMT – 2:15pm GMT

Society of Forensic Toxicologists Membership and Programs
Beth Olson, Executive Director, Society of Forensic Toxicologists

10:15am EST – 11:15am EST
2:15pm GMT – 3:15pm GMT

Are We Guilty of Being Ethically Corrupt Court Witnesses?
Dr. Nikolas Lemos, Queen Mary University of London, London, U.K.

11:15am EST – 11:30am EST
3:15pm GMT – 3:30pm GMT

Targeted High-Throughput Screening 68 Common Drugs of Abuse in Human Serum and Urine (sponsored)
Dr. Patrick Batoon, Product Manager of Triple Quadrupole LC/MS, Ion Sources, and Nitrogen Generators Agilent Technologies, Santa Clara, CA

11:30am EST – 12:00pm EST
3:30pm GMT – 4:00pm GMT

Panel Discussion
All of the day’s speakers
Monday – June 8th, 2020
9am EST – 9:15am EST
1pm GMT – 1:15pm GMT

**Cadaverous Changes & Post-Mortem Toxicology**
Federica Bortolotti, MD, PhD, Associate Professor of Legal Medicine, Unit of Forensic Medicine, Dept. of Diagnostics and Public Health, University of Verona, Verona, Italy

**Abstract:** The post-mortem toxicology is the branch of forensic toxicology specifically devoted to the search for xenobiotics in cadaverous fluids and tissues for identifying the cause and/or the manner of death.

Although the methodological approach in post-mortem toxicology is similar to that used for toxicological analyses for living persons, there are some significant differences.

Firstly, the body, after death, undergoes different modifications (cadaverous changes) which affect the physical and chemical characteristics of the biological fluids/tissues. Among these changes, the most relevant are related to the cessation of the cellular and systems activities, and to the body dehydration and decomposition. These changes pose specific issues/challenge in both tissue/fluids collection and analytical phase. Indeed, the post-mortem loss of water because of dehydration dramatically reduces the blood fluidity, thus hindering the collection of this biological fluid from the peripheral sites (e.g. inguinal fold, cubital fold) unless the body undergoes section. Moreover, the cessation of vital activities and body decomposition are responsible of the phenomenon of post-mortem redistribution of drugs. It consists in the diffusion of drugs along a concentration gradient, from sites of high concentration in solid organs into the blood with consequent artificial elevation of the drug levels. This phenomenon, although known for several years by the toxicologists, still represents a challenge in the frame of post-mortem data interpretation since it looks depending on the type of drugs and is, to some extent, unpredictable.

Another significant characteristic aspect of post-mortem toxicology is the higher number of analyzable fluids/tissues in comparison to that available for the toxicological analyses in living persons. Indeed, during an autopsy, we can collect, in addition to the traditional matrices (i.e. blood, urine, and hair), samples of vitreous humor, bile, liver, bone, etc. This higher variety of biological samples increases the amount of information provided by the toxicological analyses. However, it also poses significant interpretation issues mainly concerning the administration/consumption time of the identified xenobiotics and concerning the role of these substances in the cause/manner of death.

Finally, it is worth mentioning that usually the autopsy and related procedures (e., gross organ examination, sample collection, etc.) are unique (not repeatable) since the deceased, after the autopsy is buried or, often, cremated. It makes necessary to think in advance what kind of samples we are interested in. Moreover, it will be necessary to collect the samples in an amount enough to carry out the analyses and to save a part for possible future further tests.

The above-mentioned peculiar aspects of post-mortem toxicology explain the need for a strict collaboration between the pathologist and the toxicologist mostly in post-mortem cases in which the toxicological aspects look relevant.

**Detailed Learning Objectives:**

- a) One will learn the main cadaverous changes, which affect the post-mortem sample collection, the analysis of cadaverous fluids and tissues, and data interpretation
- b) One will learn the main aspects of the important phenomenon of post-mortem distribution of drugs in its both physio-pathological and interpretative aspects
- c) One will learn the characteristics of the different biological matrices suitable for post-mortem toxicology.
Kratom and Mitragynine in Forensic Casework
(sponsored)
Dr. Barry K. Logan, PhD, D-ABFT, Chief Scientist, NMS Labs

Abstract: Mitragynine is the primary active drug in the leaves of the tropical tree Mitragyna speciosa. Preparations and extracts of the leaves have historically been used for their psychopharmacological effects in parts of Africa and Southeast Asia, but have recently gained popularity in the United States. Mitragynine is one of 25 different alkaloids in the Kratom plant, and is considered to be the primary active alkaloid, making up approximately 60% of its alkaloid content.

The effects of typical recreational doses of mitragynine are reported to include euphoria, relaxation, increased energy, analgesia and sensory enhancement. At higher doses the drug is a µ-opioid agonist with analgesic effects and is gaining increasing use to aid as an adjunct to opioid use in the treatment of chronic pain, and is also used in opioid cessation therapies to minimize the effects of withdrawal.

In the US currently, kratom, and mitragynine are not regulated or scheduled. The toxicity of the drug is widely debated, with strong advocates for maintaining its unregulated status as a supplement, while in many regions of the world where it has been used for a much longer time, it is considered a dangerous drug and is scheduled alongside opioids in these countries.

The analysis of mitragynine is challenging due to the number of related compounds present in the plant, and the fact that the drug has three chiral centers, with up to eight diastereomers, all with similar mass spectra. In addition, mitragynine has poor stability at room temperature.

This presentation will discuss the pharmacology and effects of mitragynine, and review methods for its analysis, and present concentration and other drug co-morbidity data from impaired driving arrests, and death investigation cases. It will describe the range of effects documented in suspected impaired drivers and the circumstances and cause of manner of death from postmortem casework.

Detailed Learning Objectives:
1. Be able to describe how Kratom encompasses a wide range of uses - supplement, stimulant, sedative, opioid withdrawal agent, addictive psychoactive substance, and toxic death.
2. Be able to assess and select optimum methods for the analysis of the Mitragynine, the active component of kratom, in forensic toxicology casework
3. Apply knowledge about the effects of Mitragynine to the interpretation of death investigation and impaired driving casework
Studies on (Time-Dependent) Postmortem Redistribution - Application of CT-guide Biopsy Sampling

Dr. Andrea Steuer, Department of Forensic Pharmacology and Toxicology, Zurich Institute of Forensic Medicine, University of Zurich

Abstract: During the forensic postmortem investigation into the cause and manner of death, a forensic toxicologist aims to determine a legal or illegal drug intake or application prior to death and attempts to assess the contribution of a drug towards the cause and manner of death. The key concept in this context is whether or not the concentration of a drug in a postmortem sample accurately reflects the concentration at time of death. Besides antemortem- (reference values from living people) and perimortem factors (agonal phase), particularly postmortem factors may influence case interpretation. The anatomical and physiological changes that can alter drug concentrations artificially after death are summarized in the term postmortem redistribution (PMR). Caused by diffusion processes, degradation or drug neo-formation driven by microorganisms, significant site- and time-dependent variations in drug concentrations may be observed compared to time of death. Various studies report, that pH, volume of distribution (Vd), protein binding affinity, bacterial biotransformation action and lipophilicity may, upon others, influence the extent of PMR. Studies performed mainly focused on site-dependent changes. Generally, the predominant tool to predict the extent of PMR is the cardiac-to-femoral blood concentration ratio (C/P-ratio). As an alternative approach the liver-to-femoral blood (L/P-) ratio is used to estimate the extent of PMR. However, still conflicting data exist concerning human PMR. Contradictory findings reinforce the doubts, that site-dependent concentration differences or C/P ratios measured at autopsy can actually predict time-dependent redistribution. So far, only few studies looked at time-dependent changes in femoral blood or central blood. If at all, they were mainly performed in animal models, where sampling could be performed at defined times after death indicating that that the most important quantitative changes occur very rapidly during the first 24 h. Recent systematic human studies in Australia were performed sampling peripheral blood at two time points, at admission of the body in the mortuary and at autopsy. This study on PMR of antipsychotic drugs revealed massive changes in drug concentration with increasing postmortem interval in both directions. The Zurich Institute of Forensic Medicine has an internationally unique sample collection workflow, utilizing a computed-tomography guided biopsy tool that allows minimal invasive sampling of human body fluids (peripheral and central blood, urine) and tissue samples (lung, liver, kidney, muscle (thigh), spleen) at different time-points without interrupting postmortem processes. This workflow allows combined time-dependent PMR studies not only in blood, but also in alternative matrices. Until now, together 200 data points from the 30 highest prevalent drugs in Switzerland (including morphine, methadone, fentanyls, benzodiazepines, antidepressants and antipsychotics) were collected and indicated PMR to varying extent in all analyzed matrices depending on the drug.

Detailed Learning Objectives:
1. Understand the basics of postmortem redistribution
2. Have information on different predictors of postmortem redistribution
3. Have information on CT-guided biopsy sampling of postmortem samples (blood, tissues)
Tuesday – June 9th, 2020
9am EST – 10:00am EST
1pm GMT – 2:00pm GMT

Reference Postmortem Blood Drug Concentrations
Professor Henrik Druid, Karolinska Institutet, Stockholm, Sweden

Abstract: The interpretation of toxicological results in unclear deaths may often imply a challenge for forensic pathologists and toxicologists. Whereas many pathologists lack in-depth knowledge of the toxicity of certain compounds, they do make findings during autopsy and have a more detailed information from the police about the case history and circumstances surrounding death. Toxicologists will on the other hand have more experience with analytical findings, since they receive samples from many pathologists, and have the opportunity to compile the toxicological results from many cases. In addition, several forensic toxicology laboratories also carry out analysis in DUI and petty drug offence cases. However, by a closer collaboration, pathologists and toxicologists can share information and get a more comprehensive view of toxicity of drugs. The ToxicoList project has taken advantage of this and since the national forensic toxicology laboratory in Sweden has performed full toxicology even in cases with causes of death other than poisoning, we have been able to form a “postmortem control” group. This group consists of cases where it is obvious that the person was not incapacitated by drugs at the time for their sudden demise, e.g. hangings, shootings and motor vehicle drivers. The strategy allows for a comparison of the concentration ranges between certified fatal intoxications (separately compiled for mono-intoxications and multiple drug intoxications) and the postmortem controls. There are other countries with centralized forensic toxicology activities where this strategy can be applied, provided that information can be exchanged between the forensic pathology institutes/medical examiner’s offices and the toxicology laboratories. Since a few years back we have established a collaboration with the Victorian Institute of Forensic Medicine in Melbourne, which has a similar organization as Sweden and can compile large amounts of data. This is important since the data sample size is important. If a new drug is detected, then a certain number of cases, both postmortem controls, and intoxications, are needed to produce reliable blood drug reference levels. We have recently performed statistical analyses of the results of select drugs to estimate such sample sizes. Having said that, it is important that both postmortem controls and intoxication groups are formed by cases that are well characterized, i.e. that there are no major ambiguities regarding the cause of death and circumstances surrounding death. Hence, a number of inclusion criteria must be fulfilled for meaningful reference ranges. Finally, the interpretation of levels of many drugs of abuse, particularly opioids, represent a major challenge. However, there are some typical circumstances and autopsy findings that are important for the interpretation of opioid toxicity death. Segmental hair analysis for opioids and metabolite concentrations for some of them may also be helpful.

Detailed Learning Objectives:
1. Understand how postmortem reference concentrations can be generated from analytical results obtained in the forensic routine casework.
2. Gain knowledge about the number of cases that are necessary to obtain reliable reference ranges, and to what extent the required data sample size varies between different types of drugs.
3. Appreciate the problems with interpretation of the blood concentrations in opioid toxicity deaths, given the highly variable degree of tolerance among the victims.
High Resolution Screening of NPS in Postmortem Samples
(sponsored)
Sarah Olive, Mass Spec Technical Support Scientist, Shimadzu Scientific Instruments

Abstract: Designer drugs are classified as clandestinely synthesized drugs that are created with the intent to develop a substance that differs only slightly from the controlled substance chemically, but not pharmacologically (dea.gov, designer drugs). The number of new designer drugs, synthetic cannabinoids and synthetic opioids, and their availability has exploded in recent years, leading to widespread social impact on local communities. A Nexera LC-40 UHPLC coupled to an ultra-fast scanning LCMS-9030 Q-TOF mass spectrometer was used to create a tandem mass spectra library and screening method for common NPS. Fentanyl analogs and synthetic cannabinoid standards were individually run using MS/MS scan mode utilizing a rolling collision energy ranging from 18-52V to create a comprehensive NPS tandem high resolution library. The chromatographic separation provided full resolution of most isobaric/isomeric compounds with precursor exact masses less than 5 ppm between the measured and calculated values. Post mortem samples (Miami-Dade and Tarrant County) were analyzed using the screening method to look for the presence of novel psychoactive compounds. In addition, un-targeted analysis of a post mortem sample was performed using Insight Explore “Find” function. Formula prediction using MS1 on mass 364.20371 gave over 900 hits using standard searching programs. The data-dependent MS2 spectrum showed a presence of an indazole core group, which narrowed the search down to 5 structures, which was then successfully matched with a compound in the library database.

Detailed Learning Objectives:
1. Understand the benefit of high resolution mass spectrometry for toxicology screening
2. Learn how to create a high resolution mass spectra library for novel psychoactive substances
3. Understand how unknown peaks can be identified using high resolution mass spectrometry
Pharmacogenomic Testing for Forensic Toxicology

Alan WU, Ph.D., University of California, San Francisco (UCSF)

Abstract: Pharmacogenomics is the study of how genes affect an individual’s response to drugs. It is a major component of precision medicine. As demonstrated through clinical trials, the US FDA approves new drugs at dosages that are effective for the majority of enrolled subjects. Individuals with genetic variants should be given more drug (ultra-rapid metabolizers) to produce the desired therapeutic effect, while others (slow metabolizers) should be given less in order to avoid toxic effects. Genetic testing is needed to identify these individuals. For prodrugs, this situation regarding dosing optimizations are exactly reversed.

For postmortem investigations, unexpected toxicities can occur for individuals who carry genetic variances through their germ line. If there is a mismatch between the individual’s prescription and their postmortem drug levels, genotyping can be an important variable in determining their cause of death. The important pharmacogenomics targets are genes that encode the hepatic phase I oxidative enzymes (especially cytochrome P450) and phase II conjugative enzymes (especially UDP glucuronosyltransferase). More recently, variances in transporter genes have become part of an individual’s pharmacogenomics profile. These proteins affect the importation and clearance of drugs in and out of important organs, such as the kidneys. However, they have not yet been adopted into routine clinical practice.

The “opioid crisis” has led to great increased the death rate in the U.S. This abuse is a combination of pain medications and recreational drugs. Many pain management clinics order urine drug testing to monitor for therapeutic compliance and abuse of recreational drugs. While this service is effective for the majority of subjects, there are a few patients where pharmacogenomics testing can be useful.

Codeine is a prodrug and is metabolized through CYP P450 2D6 to morphine for pharmacologic action. Individuals who are poor metabolizers, e.g., CYP2D6*4 will not benefit from codeine use. Those who are rapid metabolizers, e.g., multiple copies of the CYP2D6*1 gene will produce an excess of the active metabolite. Morphine is metabolized to normorphine through CYP3A4, and to morphine-3-glucuronide and morphine-6-glucuronide through UDP-UGT1A1 and 2B7. Variances in these enzymes affect therapeutic concentrations of morphine. Similar reactions occur for hydrocodone to hydromorphone and oxycodone to oxymorphone. Heroin is metabolized to 6-monoacetyl morphine and then to morphine through carboxyesterases. Some individuals who do not produce morphine from 6-MAM, although the exact pharmacogenomics mechanism is unknown. Synthetic opioids such as fentanyl and tramadol also undergo transformations using these same enzymes.

Pharmacogenomic testing can also be useful to determine cause of death for those presenting with toxic epidermal necrolysis (Stevens Johnson Syndrome). Drugs such as abacavir, carbamazepine, allopurinol and others, are linked to variants in the B-family of human lymphocyte antigen (HLA).

DNA can be extracted from postmortem leukocytes or excised tissues. Pharmacogenomic testing is performed following nucleic acid extraction and amplification using polymerase chain reaction. Targeted gene SNP arrays are available commercially for detection of variants. There are reference laboratories that specialize in postmortem pharmacogenomics testing (e.g., Ambry Genetics, Aliso Viejo, CA).

Detailed Learning Objectives:
1. Understand how pharmacogenomic testing can be used to select therapy for optimum efficacy and toxicity avoidance.
2. Know how pharmacogenomics testing can be used to assist in cause of death investigations.
3. Understand genetic variance in CYP26 and its affect on the pharmacogenomics of opiates.
Ultrafast Screening of 263 Drug Targets in Blood (sponsored)
Kevin McCann, Product Owner for RapidFire and StreamSelect, Agilent Technologies

Abstract: A common approach to the analysis of postmortem blood for drugs includes an initial screen by ELISA. While able to quickly detect the presence of classes of drugs, an ELISA screen cannot differentiate between the individual drugs within those classes. To confirm the presence of specific drugs, laboratories often follow ELISA screening with something like LC/TOF analysis. In order for the LC/TOF analysis to be effective in this application, chromatography must be used to separate isobaric compounds and interferences. It is not uncommon for this chromatography to be in excess of 10 minutes, potentially creating a bottleneck for sample throughput.

A method has been developed for the ultrafast screening of drug targets utilizing the Agilent RapidFire High-Throughput MS System coupled to a 6545 LC/Q-TOF (RF/Q-TOF). This approach utilizes the unique characteristics of the RapidFire and Q-TOF to increase sample throughput. Unlike a LC/TOF, LC/Q-TOF instrumentation is able to filter ions of a specific mass and fragment them in a collision cell before performing time-of-flight analysis to collect high resolution mass spectra. Fragmenting a precursor ion creates a spectra of product ions and provides an extra level of specificity to the data. This specificity is further increased by collecting data at multiple collision energies supplied during the analysis. In this particular application, the added specificity in the acquired data eliminates the need for chromatography to separate isobaric compounds, allowing the RapidFire to introduce samples at a rate of 10 seconds per injection.

In order to identify drug targets present in samples, acquired data is compared to data in a Personal Compound Database Library (PCDL). The PCDL contains an accurate mass database and an accurate mass MS/MS spectral library, populated with known high-quality spectra for the analytes of interest. For this application, database scores were calculated with a mass tolerance of 10 ppm and accounted for 50% of the overall score for an individual target. Library scores were also calculated for the fragmentation spectra at 10, 20, and 40 volts. The three library scores for each target were averaged and accounted for the remaining 50% of the overall score.

Once the data acquisition and analysis approach was determined, drug-free (blank) whole blood was spiked with a subset of drugs commonly screened for by ELISA. The blank and spiked whole blood were both extracted using a supported liquid extraction protocol and analyzed using the RF/Q-TOF method. Data was reviewed to determine the collision energies that provided unique fragmentation spectra for the analytes of interest. The calculated scores for the spiked drugs were high enough to ensure true positives and avoid false positives. Some collision energies produced non-unique fragment spectra for a small number of targets. By excluding these collision energies for the affected targets, it was also possible to eliminate false negatives. This confirmed that the added specificity provided by the MS/MS data acquired by the Q-TOF was sufficient to replace the chromatographic separations used in the LC/TOF approach. The method was then tested by analyzing several previously screened postmortem blood samples; these samples had already been analyzed by LC/TOF with a target scope of 263 analytes. The RF/Q-TOF method was able to identify all 121 analytes that were identified by the LC/TOF method, resulting in zero false negatives.

Detailed Learning Objectives:
1. Be able to describe existing approaches to postmortem blood drug screening and their potential drawbacks.
2. Understand how the added specificity offered by Q-TOF instrumentation can improve workflows.
3. See how a spectral database and library can uniquely identify target analytes in a complex matrix.
Molecularly Imprinted Polymers for Detection of Drugs of Abuse
Miss Fabiana Grillo, University of Leicester, Leicester, LE1 7RH, United Kingdom

Abstract: Over the last fifteen years, the number of deaths from drug overdoses has increased significantly. According to the World Health Organisation (WHO), opioids were responsible for 160,000 of the 450,000 deaths from overdoses related to drug use in 2015. For this reason, there is a rise in demand for a rapid, easy to use and relatively inexpensive method of detection for drugs of abuse. The research presented here utilises molecularly imprinted polymers (MIPs) as recognition elements for drugs of abuse, with particular focus on fentanyl. Multiple optical detection platforms have been explored; utilising FRET, LPG optical fibre and fluorescently labelled nanoparticles to achieve both single analyte and multiplexed assays. A molecularly imprinted nanoparticle assay (MINA) was capable of quantifying concentration of fentanyl from 7.4 ng/mL to 598.2 ng/mL, with a detection limit of 7.38 ng/mL. The novel assay format uses the magnetic field generated by magnetic inserts on the bottom of a modified 96-microtiter plate. Displacement and competitive formats of MINA were developed, with competition achieved through use of iron oxide nanoparticles (IO-NPs) conjugated with fentanyl. Motivated by increasing throughput of sample screening, MINA was also adapted to a multiplexing assay for the simultaneous detection of cocaine and fentanyl using a displacement format.

Detailed Learning Objectives:
1. Learn about the principles of molecular imprinting technology
2. The application of nanoMIPs in different assay formats and sensors and have a broader view of the variety of molecules that can be imprinted
3. Highlighting the impact that this technology could have in the future of forensic toxicology
Automated Determination of Phosphatidylethanol (PEth) from Dried Blood Spots (DBS) (sponsored)

Oliver Lerch, PhD, Product Manager Automated Sample Preparation, GERSTEL GmbH & Co. KG, Eberhard-Gerstel-Platz 1, 45473 Muelheim, Germany

Abstract: Alcohol consumption markers can reveal alcohol uptake over a prolonged period of time. Such parameters are important in driver aptitude tests, transplant medicine, workplace drug testing and the treatment of alcoholism. While ethylglucuronide in urine or hair is an established marker, phosphatidylethanol (PEth) is novel and able to detect alcohol consumption up to 12 days after a single uptake. PEths are ethylated phospholipids where PEth 16:0/18:1 and PEth 16:0/18:2 are the most prevalent ones. Since concentrations of PEths in liquid blood are prone to change due to enzyme activity the determination from dried blood samples is preferred.

The dried blood spot (DBS) sampling technique has been developed by Guthrie in the 1960’s for diagnosis of congenital metabolic disorders in newborns. Drops of blood are collected on a special cellulose card. This format offers unique advantages over liquid blood regarding sampling, shipment, handling and storage. In the 2000s with the advent of sensitive LC- and GC-MS instrumentation it gained attention also in other areas of bioanalysis, such as pharmacology, forensic toxicology, clinical- and anti-doping analysis.

The manual extraction workflow is rather tedious: a defined area of the blood spot is punched out and extracted with an appropriate solvent in a vial or well plate. Extracts are centrifuged or filtered and further sample preparation steps such as solid phase extraction (SPE), solvent exchange or derivatization can follow before LC/MS or GC/MS analysis. Automation of dried blood spot extraction has been developed in recent years. In this study a GERSTEL Dried Blood Spot Autosampler (DBS-A) basing on the MultiPurpose Sampler (MPS) has been employed. The blood spot card simply needs to be positioned on the autosampler rack and all subsequent steps, including chromatographic/mass spectrometric analysis, are performed automatically. After recognition of the blood spot by an integrated camera, a 2, 4, 6 or 8 mm clamp is used to make a leak free seal that allows a solvent to flow through the card and extract analytes. The system also has the option of adding an internal standard during this process. Cartridge based SPE cleanup can be automatically performed on the extract using the GERSTEL SPEXos system followed by direct injection into an LC-MS/MS instrument. Alternatively, the extract can be collected in vials or well plates with or without SPE cleanup. Automated solvent exchange or derivatization can also be added if required. Both techniques, the online DBS-SPE-LC-MS/MS and the DBS-SPE-Collection workflow, have been employed in the present application. A possible extension to the analysis system is an NIR spectrometer from Buchi which allows the automated and non-destructive measurement of hematocrit values of dried blood spots on DBS cards before analysis.

Blood samples were collected using HemaXis DB 10 devices (DBS System). They incorporate a microfluidic chip for accurate and precise volume sampling (10 µL), a standard cellulose card (Whatman 903 Protein Saver) and a protective polymer envelope. The credit card sized device provides a practical solution for whole blood collection from a fingertip that can be performed anywhere at any time. Full spot analysis enables results with minimized hematocrit bias, a topic discussed in scientific literature.

At first an online DBS-SPE-LC-MS/MS workflow was developed. The blood spot was desorbed by a mixture of water, acetonitrile, isopropanol and formic acid. Analytes were trapped in the SPEXos on a silica-based C18 sorbent. After washing the SPE cartridge and the DBS-A clamp the LC (Agilent 1260) eluent stream was directed through the cartridge whereupon analytes were transferred onto a silica-based C18 column. Separation was achieved by a gradient consisting of water/formic acid and acetonitrile/ammonium acetate followed by mass spectrometric detection with an Agilent 6460 triple quadrupole mass spectrometer. Multi-reaction monitoring (MRM) transitions were recorded for the two analytes (PEth 16:0/18:1, PEth 16:0/18:2) and their two corresponding deuterated internal standards. Analytes and interferences were well separated.

Limits of quantification for both analytes were below 10 ng/mL as well as relative standard deviations of repeat analyses below 10%. Besides real samples taken by the HemaXis device, external reference samples were analyzed successfully. Furthermore, a DBS-SPE-Collection workflow including automated evaporation and reconstitution in LC compatible solvent has been developed. Both methods proved to be accurate, precise and reliable while manual labor is reduced to simply putting the DBS cards onto the autosampler rack.

Three (3) Detailed Learning Objectives:

After having attended this presentation, one will

a) have an understanding of phosphatidylethanols (PEths) as novel alcohol consumption markers and their significance in forensic toxicology and other areas of application.

b) understand the principles of the dried blood spot (DBS) sampling technique, its applications and automation possibilities.

c) gain insight into current automated DBS analysis methods for PEths.
Abstract: High-resolution mass spectrometry is an incredibly powerful tool in modern forensic toxicology. This is especially true when it comes to identifying novel psychoactive substances including fentanyl derivatives and synthetic cannabinoids. However, the technology does not offer an analytical panacea. The nature of forensic casework means that care must be taken when interpreting analytical data. Fortunately, by taking into consideration some of the nuances of toxicological analysis, it is possible to design data acquisition methods that mitigate against, and help overcome some of these problems. This presentation will include examples of (i) the importance of thorough chromatographic separations, (ii) the use of isotopes to ‘extend’ calibration and select unique ions for extraction, and (iii) minimizing risks of false negative results by careful library building.

Detailed Learning Objectives:

a) Benefits of the use of high-resolution mass spectrometry in forensic toxicology
b) Pitfalls when using this technology
c) Some approaches to mitigating the risks and avoid the pitfalls associated with this technology
Routine Screening of Drugs in Whole Blood Using LC/Q-TOF (sponsored)
Dr. Karen E. Yannell, LC/MS Application Scientist Agilent Technologies, Santa Clara, CA

Abstract: High resolution mass spectrometry provides the confidence we need to correctly identify drugs in complex matrices. However, when screening for hundreds or thousands of drugs, implementing this technology in high throughput laboratories is challenging due to the lack of routine data analysis tools. Discussed here is a seamless workflow using a new LC/Q-TOF with much improved resolution, dynamic range and mass accuracy and the Screener software tool for data analysis.

A whole blood sample spiked with over 150 drugs was automatically processed using an automated liquid handling system. A ten-minute reversed phase chromatography method separated analytes with consistent retention times. Molecular ion and fragment ions were detected in a data independent fashion. A routine analysis workflow was created in MassHunter Quantitative Analysis software allowing each sample to be processed and analyzed in only a few minutes. Spiked samples showed good reproducibility and low detection limits. The mass accuracy of the analyte’s molecular ion and fragment ions was consistently below 5 ppm. These software improvements are allowing the LC/Q-TOF or LC/TOF to be deployed in routine forensic and toxicology laboratories.

Detailed Learning Objectives:
1. Understand how to use LC/Q-TOF in data independent mode for drug screening.
2. How to set up an analysis method and how new software tools can make Q-TOF analysis routine
3. Complete workflow from sample prep to reporting for high throughput drug screening using an LC/Q-TOF
Cannabis And Cannabis Derived Products: Hot Topics Likely to Influence Your Practice As A Forensic Scientist.

H. Chip Walls, Forensic Analytical & Clinical Toxicology Consultant and Training Specialists, Miami, FL, USA

This 1 Hour eagle eye overview of cannabis and cannabis products will address:

- The Farm Bill and Its Impact on the Criminal Justice System
- A concern common to law enforcement and prosecutors – the difference between hemp and cannabis
- A brief overview of the Cannabis plant
- Content variation in cannabis thus in cannabis derived products
- Does POTENCY apply to Pot?
- Various forms of cannabis and cannabis products including the nonsense that a “dose” of THC exists
- How the use of Cannabis has changed in the last 10 years?
- New methods of use: vaping, dabbing, tampons
- Delta-8 THC as a Drug of Abuse and as an Interferent in Methods for Delta-9-THC and metabolites
- Isomers of Cannabinoids
- Potential or theoretical impact of delta-8-THC “interferent”
- How to distinguish use of CBD products and use of CBD + other Cannabinoids
- Cross-reactivity of CBD or metabolites with THC-COOH screening assays. Seems like a lot is not known about this. Are you seeing “false positive” initial tests all the time with THC-COOH “failed to confirm” but Carboxy just below the “cutoff” and the person claimed use of CBD products or a D-8 vaper.
- Do test methods for CBD cause conversion to THC-COOH?
- FDA Regulation of Cannabis and Cannabis-Derived Products, Including Cannabidiol (CBD)
- What is the recommended DOSE of CBD?
- CBD “products”:
  - The analytical challenges around CBD and THC analysis in products and biological fluid
  - Hemp seed oil vs. Hemp oil
  - Does the product label tell the truth?
  - U.S. FDA approved - Epidiolex®, Marinol®, Syndros®, and Cesamet®
- What is the best biological specimen for the investigation of impaired drivers?
  - Just getting in a tube of blood and testing has become routine or maybe you thought so-in pursuit of the last molecule
  - The holy grail-- grey stopper vacuum tube
  - Living versus deceased case investigations
  - Antemortem specimens
- Postmortem “blood” the Enigma
- Roadside drug testing-Oral fluid or Breath or FIT (Pharmacodynamics)
  - Oral fluid THC measurements – what if a person is taking CBD orally? Can you distinguish
  - Cannabis use as a “Cause of Death”
- Pot and your heart
  - The responsibility of the Forensic Toxicologist is to interpret the “number”-right?”
  - Can we relate a “blood” cannabinoid concentration to brain receptor occupancy?
- What is more important pharmacokinetics or pharmacodynamics”
  - THC, CBD, drug and herb interactions.... How does that change your interpretation?
- The medical and recreational cannabis debate.
- Medicalization or Legalization?
  - Cannabis Use During Pregnancy and lactation
Thursday – June 11th, 2020
10:00am EST – 10:15am EST
2:00pm GMT – 2:15pm GMT

Discovery of NPS in Forensic Toxicology Casework
(sponsored)
Dr. Alex J. Krotulski, Research Scientist, Center for Forensic Science Research and Education (CFSRE), Willow Grove, PA USA

Abstract: Novel psychoactive substances (NPS) are responsible for increased morbidity and mortality as new drugs emerge on a weekly to monthly basis. NPS identification are generally underestimated or underrepresented due to lack of testing or knowledge about new drugs. The lack of centralized programs to accurately track and monitoring NPS identifications or intoxications make it difficult for forensic toxicologists to remain abreast to synthetic drug related trends or issues. As a result, NPS Discovery (www.npsdiscovery.org) has developed a program to evaluate forensic toxicology samples for emerging NPS not previously identified in the biological samples and to disseminate information to national and international stakeholders. Remaining at the forefront of NPS trends is critical to tackling analytical and interpretative issues in forensic toxicology.

As a general workflow, CFSRE receives extracts of blood and urine samples, correlating to cases of suspected NPS use, from NMS Labs for re-analysis (i.e. sample-mining). This process allows for direct discovery of analytes not targeted within the initial scopes of testing (e.g. new synthetic drugs). Extracts are analyzed using a Sciex TripleTOF® 5600+ quadrupole time-of-flight mass spectrometer coupled with a Shimadzu Nexera XR ultra high performance liquid chromatograph (LC-QTOF-MS). SWATH™ acquisition is used for isolation of product ions following the acquisition of precursor ions by TOF MS scan. In addition to NPS discovery, this non-targeted analytical approach allows for complex drug characterization (e.g. combinations, metabolism, etc.).

Since 2018, more than 65 NPS have been identified for the first time nationally or internationally through our NPS Discovery program. Of these drugs includes 16 opioids, 6 opioid precursors, 13 synthetic cannabinoids, 17 stimulants, 9 hallucinogens, and two benzodiazepines. Success stories include the identification and dissemination of information regarding the emerging NPS 4F-MDMB-BINACA and isotonitazene. Both were identified through NPS Discovery for the first time in the United States. In early 2020, isotonitazene is the most prominent NPS opioid on the market, while 4F-MDMB-BINACA is the second most prevalent synthetic cannabinoid. Due to the rapid proliferation of emerging NPS, public health alerts are often issued to address the lack of awareness, their involvement in forensic and clinical casework, and the need for inclusion in testing scopes.

Forensic toxicologists should be aware of emergent NPS, as drug-specific popularity continues to increase. Laboratories should consider addition of emerging NPS to blood and urine testing procedures, incorporating metabolites when known and/or available. Novel procedures for the discovery of drugs and/or NPS missed during standard targeted analysis should be more widely implemented in forensic toxicology.

Detailed Learning Objectives:
1. Attendees will learn about early warning systems (EWS) and programs that focus on the dissemination of toxicologically relevant information.
2. Attendees will understand the ever-changing landscape of NPS within forensic toxicology samples and the classes of drugs encountered among death investigation casework.
3. Attendees will learn about the impact of NPS and programs focused on disseminating relevant information to public health and public safety.
Pharmacology and Toxicology of Synthetic Cannabinoids

Prof. Dr. Volker Auwärter, Forensic Toxicology, Institute of Forensic Medicine, Medical Center – University of Freiburg, Freiburg, Germany

Abstract: Synthetic cannabinoids, more precisely synthetic cannabinoid receptor agonists (SCRAs) first appeared as drugs of abuse on a greater scale around 2008 in Europe. Many of these drugs show extraordinarily high potency at the CB1 receptor and severe, sometimes lethal toxicity. New compounds are introduced into the market with high frequency to circumvent legal restrictions and/or detectability in biological samples, adding up to more than 200 SCRAs of various structural classes monitored by the European Monitoring Centre of Drugs and Drug Addiction (EMCDDA). In clinical and forensic toxicology the detection of these drugs or their metabolites has become a major challenge which is made even more difficult by the unavailability of reference standards. In particular for urine analysis, the main metabolites have to be identified as analytical targets for screening methods. Strategies to identify suitable consumption markers include assays based on pooled human liver microsomes (pHLM) or hepatocytes. Target analysis is usually performed by LC-MS/MS or LC-HRMS to reach the low LODs needed due to the high potency (and low target concentrations) of these drugs. Another task is to characterize the interaction of the compounds with the endocannabinoid system by measuring receptor binding and receptor activation. While receptor binding is usually characterized by competitive binding assays involving radioactively labelled ligands, a multitude of assays is available for measuring receptor activation by SCRAs, among them [35S]GTPγS assays, assays based on β-arrestin 2 recruitment, and cAMP signaling assays.

Detailed Learning Objectives:
1. Have an overview of the development of the phenomenon of SCRAs in the last ten years
2. Know the analytical strategies for the detection of SCRAs (and their metabolites) in biological samples
3. Know the principles of assessment of biological activity and pharmacological potency of SCRAs
Abstract: Forensic analyses, ranging from white powder drugs to blood alcohol, generates a large number of samples. Instrument downtime and increased sample load can result in a significant backlog forcing analysts into working overtime. There are many features of the Intuvo GC that can improve this. The guard chip technology collects contamination before it reaches the analytical column. This means column trimming is not needed thus maintaining reproducible retention times. This allows the user to quickly return to running samples without additional retention time locking or time segment adjustments for the mass spectrometer. The Intuvo flow chips afford quick configuration of the GC especially for blood alcohol analyses that are taking advantage of dual columns or detector splitting. With Smart ID Keys for each of the flow chips the Intuvo GC will know its configuration, the operator does not need to configure the system. Employing narrow bore columns and taking advantage of Intuvo's direct heating technology allows shortened cycle times. Lastly the smart, connected features of Agilent's GCs provide maintenance demonstrations, diagnostics, and troubleshooting guidance, if needed. All of this can be accessed through a remote browser user interface using your company's VPN. In this talk we will show examples of the analytical improvements achieved using the Intuvo 9000 GC.

Detailed Learning Objectives:
1. How Intuvo can reduce or eliminate backlog in forensic labs by improving cycle time
2. The guard chip preserves retention times by eliminating column trimming.
3. How the onboard maintenance and diagnostics can facilitate more productive uptime.
Title TBA
Dr. Andrew Mubiru, Uganda Police Force

Abstract: tba

Detailed Learning Objectives:
1. tba
Society of Forensic Toxicologists Membership and Programs
Beth Olson, Executive Director, Society of Forensic Toxicologists

Abstract: The Society of Forensic Toxicologists has been providing forensic toxicologists with education and networking opportunities for 50 years, highlighted by the Annual Meeting which is attended by more than 1,000 scientists in the field each year.

Detailed Learning Objectives:
1. Understand the value of membership in a professional association
2. Gain awareness of educational opportunities offered by SOFT
3. Become familiar with SOFT’s Professional Mentoring Program
Are We Guilty of Being Ethically Corrupt Court Witnesses?
Dr. Nikolas Lemos, Queen Mary University of London, London, U.K.

Abstract: When preparing for testimony, whether in a criminal court, a tribunal, a civil trial or a pre-trial hearing, a forensic toxicologist must remain unbiased and must help the judicial system by (1) accurately representing their education, training, experience, and area of expertise, (2) being properly trained and determined to be competent through testing prior to undertaking the examination of any evidence, (3) treating evidence and specimens in such a way to always avoid tampering, adulteration, loss, or unnecessary consumption, (4) basing conclusions and opinions on generally accepted tests and procedures following current method validation standards and practices, (5) following the laboratory’s validated procedures, policies and practices, (6) adhering to career-long learning in toxicology, (7) remaining independent, impartial, detached, and objective, approaching all examinations with due diligence and an open mind, (8) undertaking full and fair examinations within the scope of our laboratory’s testing capabilities and per their policies, (9) remaining aware of their own limitations and rendering opinions within their area of expertise, (10) reporting any unethical, illegal, scientifically questionable conduct, or impaired competence, (11) raising the alarm if there is potential for, or there has been a miscarriage of justice due to circumstances that have come to light, incompetent practice, or malpractice, (12) reporting conflicts and attempting to resolve them, (13) never participating in any case on a contingency fee, (14) always presenting accurate and complete data in reports, testimony, publications and oral presentations, (15) always operating in a timely manner, (16) making and retaining full, contemporaneous, clear and accurate records of all examinations conducted and conclusions drawn, in sufficient detail to allow meaningful review and assessment of the conclusions by an independent person competent in the discipline, (17) issuing reports in which facts, opinions and interpretations are clearly distinguishable, and which clearly describe limitations of the methods, interpretations and opinions presented, (18) communicating honestly with all parties about all information relating to the analyses, (19) never altering reports or other records, or withholding information from reports for strategic or tactical litigation advantage, (20) supporting sound scientific techniques and practices and never use their positions to pressure an examiner or technician to arrive at conclusions or results that are not supported by data, and (21) only testifying to results obtained and only drawing conclusions when there is confidence that the opinions are based on good scientific principles and methods.

The presentation will discuss the above ethical guidelines and explore ways in which, either by habit or malice, they can be skewed to benefit some but not all of the parties involved in a trial.

Detailed Learning Objectives:
1. Be familiar with the ethical standards that ideally should drive the preparation and delivery or court testimony by a forensic toxicologist
2. Be aware of possible ethical traps in forensic toxicology from the laboratory to the courtroom, and
3. Have examples to avoid of questionable and possible unethical behaviors by forensic toxicologists around the world.
Targeted High-Throughput Screening 68 Common Drugs of Abuse in Human Serum and Urine (sponsored)

Patrick Batoon, Ph.D, Product Manager of Triple Quadrupole LC/MS, Ion Sources, and Nitrogen Generators, Agilent Technologies, Santa Clara, CA

Abstract: This presentation discusses a high-throughput, targeted MRM screening methodology using an Ultivo Triple Quadrupole LC/MS with a standard ESI source. The methodology was developed for the screening of 68 drugs of abuse in human Serum and Urine using a quick, 7-minute chromatographic method. The analytes in this method include a variety of different drug classes such as opioids, benzodiazepines, stimulants, and other drug classes. By utilizing the dynamic-MRM (dMRM) mode of acquisition, instrument cycle time per analyte can be maximized, since a MRM measurement will only occur during the an elution window, resulting in limits of quantitation between 100 ppt and 10 ppb, with majority of the compounds detected at the 500 ppt level.

Detailed Learning Objectives:
1. Understand a high-throughput screening methodology for toxicological screening of target drugs using tandem quadrupole LC/MS.
2. Understand the sensitivity, robustness, and general capability of the Ultivo with an ESI source.
3. Have knowledge of the curated forensics toxicology database for faster development and expansion of this MRM method. Additionally, have knowledge that users can create custom MRM databases for novel /emerging analytes not included in the database.
Prof. Simon Elliott
Director, Elliott Forensic Consulting, Ltd.

Prof Simon Elliott has over 20 years’ experience in forensic toxicology and is a Consultant Forensic Toxicologist, independent Business Consultant and Director of Elliott Forensic Consulting Ltd. He was previously the Director of Global Forensics at Alere Inc (now part of Abbott) (2017-2018) having also been the founder and Managing Director of Forensics Ltd (ROAR Forensics, subsequently Alere Forensics) in Malvern, Worcestershire, UK (2008-2017). Prof Elliott previously worked as a Clinical Scientist in the NHS at Birmingham City Hospital for over 10 years specifically involved in clinical and forensic toxicology as Section Head of Forensic Toxicology. He is a Visiting Professor in Forensic Toxicology at King's College London and holds a BSc in Biochemistry from the University of Bath and a PhD in Biochemical Toxicology (studying GHB) from the University of Birmingham. He is a Chartered Scientist and European Registered Toxicologist as well as being a member of a number of professional organisations including The International Association of Forensic Toxicologists (TIAFT, Board Member), the LTG, Chartered Society for Forensic Science, Association of Clinical Biochemistry and Royal Society of Chemistry in addition to being a founder and Chair of the UK & Ireland Association of Forensic Toxicologists (UKIAFT). An author of over 70 scientific publications, articles and book chapters, he is on the Editorial Board of Drug Testing & Analysis and Forensic Science International journals as well as being an Associate Editor of the Journal of Analytical Toxicology and an Editor of Wiley’s WIREs Forensic Science (Toxicology) and Clarke's Analysis of Drugs and Poisons. Prof Elliott has presented at many national and international meetings (including invited speaker) as well as presenting expert evidence for many years in Coronal, Civil and Criminal Court. A member of the World Health Organisation (WHO) Expert Committee on Drug Dependence, he also advises the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) and United Nations Office on Drugs and Crime (UNODC), especially regarding new psychoactive substances.
Prof. Nikolas P. LEMOS
Director, Cameron Forensic Medical Sciences, Barts and The London School of Medicine & Dentistry, Queen Mary, University of London

Dr. Lemos is a board-certified forensic toxicologist, academic and researcher who started his career researching alcohol and drug detection analysis in alternative biological specimens. After getting awarded his doctorate in Forensic Medicine and Science (Analytical and Forensic Toxicology) from the University of Glasgow in Scotland, he worked as Senior Lecturer at London South Bank University where he helped develop a new undergraduate course in forensic science. He later moved to St George's Hospital Medical School of the University of London to assume the position of Head of the Forensic Toxicology Service of its Analytical Unit where he supervised or co-supervised the research of numerous medical and graduate students who trained in the Analytical Unit.

In 2003, he became Chief Forensic Toxicologist and in 2005 Director of the Forensic Laboratory Division at the Office of the Chief Medical Examiner of the City and County of San Francisco where he spearheaded that office's ABFT accreditation.

He is actively engaged in various international professional organizations and is a Member of the Chartered Society of Forensic Scientists, Fellow of the Royal Society of Chemistry, Fellow of the American Board of Forensic Toxicology, and Fellow of the American Academy of Forensic Sciences. Dr. Lemos has consulted and testified in over 335 civil and criminal trials around the world. He has co-authored over 85 peer-reviewed papers, abstracts, and book chapters including the first ever study of Cannabinoids in Fingernails (JAT, May 1999) and the first ever study of Cannabinoids in Post-mortem Toxicology (JAT, September 2011). He has organized and hosted numerous professional scientific meetings in the USA and abroad including the 2011 Joint SOFT-TIAFT Meeting held in San Francisco with over 1,500 participants from 60 countries in attendance.

He has previously served as Science Editor of “Medicine, Science and the Law,” the Official Journal of the British Academy of Forensic Sciences, and he is the holder of many scholarships, research and equipment grants and awards. In 2005, he was honoured with a Proclamation of Achievement by the 109th Congress of the United States of America “for dedicating his life and career to community safety and social awareness,” especially regarding new psychoactive substances.
Federica Bortolotti, MD, PhD
Associate Professor of Legal Medicine, Unit of Forensic Medicine, Dept. of Diagnostics and Public Health, University of Verona, P.le Scuro 10, 37134, Verona, Italy

Federica Bortolotti graduated in Medicine at the University of Verona, Verona, Italy in 1998. At the same University she got the post-doctoral degree in Forensic Medicine in 2002 and the Diploma of PhD in Forensic Sciences in 2006. Since December 2014 she is Associate Professor of Legal Medicine at the Dept. of Diagnostics and Public Health, University of Verona. She published 75 international publications (H-index: 20) on different research topics in the fields of forensic pathology and toxicology.

Dr. Barry K. Logan, PhD, D-ABFT
Chief Scientist, NMS Labs

As Chief Scientist at NMS Labs, Dr. Logan’s leads new test design, quality, and expert testimony in forensic toxicology. He has over 150 publications, notably in novel psychoactive substances, designer opioids and synthetic cannabinoids. Dr. Logan received TIAFT award, the AAFS Rolla N. Harger Award, the National Safety Council’s Robert F. Borkenstein Award, and the Widmark Award from the International Council on Alcohol, Drugs and Traffic Safety. He served as AAFS President in 2013.
Dr. Andrea Steuer
Department of Forensic Pharmacology and Toxicology, Zurich Institute of Forensic Medicine, University of Zurich

Andrea Steuer studied pharmacy in Germany. After her degrees, she started her PhD thesis on MDMA in the Clinical Toxicology Lab of the University of Saarland. Afterwards, Andrea Steuer moved to Zurich to work at the Zurich Institute of Forensic Medicine. There she obtained her habilitation in 2018. Additionally, to her research, she contributed to service work and obtained approval as Forensic Toxicologist. In 2015 she took over the position of deputy head of the department.

Professor Henrik Druid
Karolinska Institutet, Stockholm, Sweden

Henrik Druid is a faculty professor of forensic medicine, and a senior forensic pathologist. He has also worked at the Swedish national forensic toxicology laboratory, and at the forensic toxicology laboratory at the Miami-Dade Medical Examiner’s office. His research group is carrying out both basic research on adult human neurogenesis and translational studies in the fields of forensic pathology and toxicology.
Sarah Olive
Mass Spec Technical Support Scientist, Shimadzu Scientific Instruments

Sarah Olive is a Mass Spectrometry Technical Support Scientist for Shimadzu Scientific Instruments. She has a master’s degree in Forensic Science and has spent her career utilizing LC/MS-MS instrumentation in the forensic toxicology, clinical toxicology and pharmaceutical drug discovery arenas. Prior to Shimadzu, she worked at the Dallas County Institute of Forensic Sciences for over 7 years in the forensic toxicology section.

Alan WU, Ph.D.,
University of California, San Francisco (UCSF)

Alan Wu is Chief of Clinical Chemistry and Toxicology at San Francisco General Hosp. and Prof. of Lab Medicine, UCSF, and Director of Pharmacogenomics. He is certified by the ABCC in Chemistry and Toxicology. His research interests include pharmacogenomics and clinical toxicology and has 450 publications. He has also written 5 paperback books consisting of short stories designed to promote the value of the clinical lab and pharmacogenomics to the general public.
Miss Fabiana Grillo  
**University of Leicester, Leicester, LE1 7RH, United Kingdom**

In 2016 Fabiana received her bachelor degree in biotechnology from La Sapienza University of Rome. During her studies she worked in a biotechnology group on the synthesis of PLGA based nanoparticles using a microfluidic reactor for ribavirin drug delivery systems. She moved to the University of Leicester for an Erasmus+ traineeship conducting research as part of a collaboration between the departments of chemistry and genetics, focusing on molecularly imprinted polymer nanoparticles (MIPs) as a potential drug delivery system targeting helicobacter pylori and clostridium difficile. In 2018 Fabiana has completed her masters in research at the University of Leicester, with funding awarded by the ERDF and MIP Diagnostics L.t.d. Her project concerned the development of a novel assay format to detect molecules of abuse, with particular focus on fentanyl, using molecularly imprinted polymers in collaboration with the Scottish Police and Crime Unit. Fabiana has been awarded of a EPSRC scholarship and she is in her second year of her PhD with the purpose of further pursue and expand the objectives of her MPhil project. She is currently working on the development of different detection platforms for the detection of a range of drugs of abuse.

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**Kevin McCann**  
**Product Owner for RapidFire and StreamSelect, Agilent Technologies**

As a Product Owner at Agilent, Kevin helps develop and implement high-throughput LC/MS technologies including RapidFire and StreamSelect. He has also worked as an application scientist, developing and supporting a variety of LC/MS applications. Prior to working at Agilent, he was the laboratory manager of the biochemical genetics laboratory at Mount Sinai Hospital in New York where he also developed methods for the analysis of acylcarnitine profiles, amino acid profiles, and TDM of tamoxifen.
**Dr. Lewis Couchman**  

Dr. Lewis Couchman is the Facility Director at ASI, and leads a team of scientists with a passion for developing novel analytical techniques, in particular those involving chromatography and mass spectrometry. Prior to joining ASI, Lewis worked as a clinical scientist in the Departments of Toxicology and Clinical Biochemistry at King’s College Hospital, London. Lewis is currently Vice Chairman of the London Toxicology Group.

**Oliver Lerch, PhD**  
**Product Manager Automated Sample Preparation, GERSTEL GmbH & Co. KG, Eberhard-Gerstel-Platz 1, 45473 Muelheim, Germany**

Oliver Lerch received his PhD in Analytical Chemistry in 2003. After a postdoc stay at the Max-Planck-Institute of Molecular Plant Physiology he joined GERSTEL in 2005. He has played a key role in the development of numerous products, technologies and strategic applications. In 2020 he has been appointed Product Manager Automated Sample Preparation. Oliver has over 20 years of experience in chromatographic and mass spectrometric techniques with a focus on innovative automated sample preparation.

**Dr. Karen E. Yannell**  
**LC/MS Application Scientist Agilent Technologies, Santa Clara, CA**

Karen is an expert in small molecule high throughput analysis by triple quadrupole and quadrupole time-of-flight (Q-TOF) mass spectrometry for applied markets. Her recent work has focused on developing routine Q-TOF workflows for both expert and non-expert users alike. She received her PhD in Analytical Chemistry from Purdue University under the advisement of Prof. R. Graham Cooks. Outside of the laboratory, she enjoys cooking unique dishes and hiking along the beach with her dog.
H. Chip Walls
Forensic Analytical & Clinical Toxicology Consultant and Training Specialists, Miami, FL, USA

H Chip Walls received his B.S. from the University of Alabama at Birmingham in 1972 during which time he was a research associate in the Department of Chemistry. His professional career has covered more than 45 years including 17 years at the University of Miami Miller School of Medicine, and 21 years with the Alabama Department of Forensic Sciences-Birmingham Division toxicology section and Onondaga County Medical Examiner’s Office toxicology laboratories. His experience encompasses post-mortem forensic toxicology, clinical toxicology, probation urine drug testing, drug facilitated crimes and driving under the influence cases. Currently, he is the Chief Wizard at the Forensic Analytical & Clinical Toxicology Laboratory Consulting & Training Specialists in Miami. In addition, he is a technical consultant to the forensic chemistry section of the chemistry department at Florida International University, Miami Police Department Police Academy adjunct instructor and Subject matter expert for police departments in Miami-Dade County serving the Impaired Drivers program. An active member of several toxicology organizations, he has chaired national committees and has organized numerous workshops on many aspects of the principals and practices of forensic toxicology for annual meetings of various professional organizations. He has been an invited speaker, nationally as well as internationally, on drug detection in pregnancy, the role of toxicology in prosecuting impaired drivers, and information resources in forensic toxicology, marijuana, antidepressants, anti-epileptics, psychotropic medications, narcotics, sedative-hypnotics, Field Impairment Testing and Driving Under the Influence of Intoxicants and alcohol. He has had work published on such topics as cocaine, marijuana, and benzodiazepines and forensic toxicology in peer-reviewed scientific journals or books, and presented at national forensic science meetings. He has served the Society of Forensic Toxicologist (S.O.F.T.) as Past-President (1997), President (1996), Vice-President (1995), Board of Directors (1991-1994), the Executive Board (1995), and the Editorial Board of ToxTalk (1994-present). In addition, he has served on or chaired the following committees: Driving Under the Influence of Drugs (Chair), Meeting Resources (Chair), Joint Committee on Education and Training in Toxicology (JCETT) and Health/Safety. He was S.O.F.T. Special Issue Guest Editor of the Journal of Analytical Toxicology (1992). In 2006, he was presented the Ray Abernathy award by the American Academy of Forensic Sciences Toxicology section as “Recognition of an Outstanding Forensic Toxicology Practitioner”. In 2009, he was recognized as DRE Ambassador by the Drug Evaluation and Classification (DEC) Program of the International Association of Chiefs of Police. In 2012, he was recognized by Miami-Dade MADD with it highest honor the “Heart of MADD” award for his dedicated service to public safety endeavors; In 2017, he was recognized by the National Highway Safety Administration, one of sixteen in the United States, for his toxicological expertise while serving the public safety community in their efforts remove the impaired driver from our roadway’s. He is a member of the executive board of the National Safety Counsel’s Committee on Alcohol and other drugs now known as The NSC Alcohol, Drugs and Impairment Division. His eccentricity is widely known to fellow toxicologists concerning his collection of information about the principals and practices in the field of analytical, clinical and forensic toxicology. He serves as a laboratory inspector for the National Laboratory Certification Program: Federal Workplace Forensic Urine Drug Testing 1990 to Present (SAMSHA).
Alex J. Krotulski, PhD
Research Scientist, Center for Forensic Science Research and Education, Willow Grove, PA

Dr. Alex Krotulski serves as a Research Scientist at The Center for Forensic Science Research and Education, overseeing the collaborative efforts of NPS Discovery, a flagship program for the identification of new synthetic drugs and the dissemination of information surrounding their impact. Dr. Krotulski works in the areas of forensic toxicology and forensic chemistry, handling both biological samples and seized drug materials from receipt through analysis to reporting and data curation.

Prof. Dr. Volker Auwärter
Forensic Toxicology, Institute of Forensic Medicine, Medical Center – University of Freiburg, Freiburg, Germany

Besides reading for medical students and being an expert witness in court, Prof. Auwärter has coordinated several EU projects on synthetic cannabinoids. His research also covers other new drugs, post mortem toxicology, drug metabolism and pharmacokinetic studies, and resulted in more than 150 research articles in scientific journals with peer review. He was awarded with the TIAFT Achievement Award in 2013 and received several national awards for his contributions to forensic toxicology.
Rebecca Veeneman, PhD
Applications Chemist Manager, Agilent Technologies, Wilmington, DE

Becky Veeneman is an Applications Chemist Manager in GC Marketing at Agilent Technologies. She currently leads a team of applications chemists charged with developing technical marketing material for new gas phase product introductions. She has over 15 years of research experience in gas chromatography. Becki holds a B.S. in Chemistry from Xavier University (Cincinnati, OH) and a M.S. and Ph.D. in Chemistry from the University of Michigan.

Beth Olson
Executive Director Society of Forensic Toxicologists

Beth Olson is the Executive Director of the Society of Forensic Toxicologists in Mesa, AZ. She has served in this role since 2016. Previously, Ms. Olson was the Executive Director of Temple Emanuel of Tempe, providing leadership, financial management, and strategic direction to the 450-member families. From 2004-2013 she was Director of Education Programs for Childsplay, an internationally renowned theater company for young audiences and families. Earlier in her career, Ms. Olson worked as a high school teacher and a freelance journalist. Ms. Olson has a Bachelor’s Degree in Secondary Education and English, and an MBA with an emphasis in Leadership from Arizona State University.
Patrick Batoon, Ph.D
Product Manager of Triple Quadrupole LC/MS, Ion Sources, and Nitrogen Generators Agilent Technologies, Santa Clara, CA

With a background in ion chemistry from University of the Pacific in the university’s Mass Spectrometry Core Facility, Patrick started his career at Agilent as an R&D chemist, working in the core hardware development team of the Ultivo triple quadrupole LC/MS (LC/TQ) and the MSD/iQ single quadrupole LC/MS. Through this, Patrick became the product manager of Agilent’s Triple Quadrupole LC/MS portfolio managing the Ultivo LC/TQ, 6470 LC/TQ, and 6495 LC/TQ while managing the supported Ion Sources and N2 generators for his product lines.
Detection and Quantitation of Nine Fentanyl Analogs in Urine and Oral Fluid Using QSight® Triple Quad LC/MS/MS

Yiling Ke1, Jenna Gardner1, Joseph Cox2, Colby Ott2, Joseph Jones3, Frank Kero3, Sabra Botch-Jones1

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Introduction: Fentanyl is a synthetic opioid analgesic and has resulted in an increasing number of drug overdose since 2013 and has contributed to the opioid epidemic in the United States. In addition, fentanyl analogs, originally used as analgesics or animal tranquilizers, have emerged in the drug market. Fentanyl and its analogs, similar to other opioids, work as full B-agonists, binding with B-receptor in the brain. Fentanyl and its analogs elicit more potent effects compared to the traditional opioids (e.g., morphine or heroin) being abused. With the emergence of fentanyl analogs, identifying and differentiating those analogs becomes a challenge due to their structural similarity to fentanyl.

Objective and Methods: The purpose of this research was to develop a method of identifying and quantifying nine fentanyl analogs in urine and oral fluid using the QSight™ Triple Quad LC-MS/MS (PerkinElmer, Waltham, MA, USA), coupled with a Halo C18, 2.7um column (Advanced Materials Technology, Wilminton, DE, USA). The method was validated based on Academy Standards Board (ASB) Standard 036, Standard Practices for Method Validation in Forensic Toxicology. The analytes in this research included fentanyl, norfentanyl, acetylfentanyl, carfentanil, cyclopropylfentanyl, methoxyacetylfentanyl, valeryl fentanyl, furanyl fentanyl and 4ANPP. All samples, calibrators, and QCs were prepared by spiking certified reference standards (Cerilliant Corporation, Round Rock, TX, U.S.A) into donated human urine or human oral fluid. The samples were prepared using supported-liquid extraction (SLE). SLE was performed using ISOLUTE® SLE+ 1mL columns (Biotage AB, Uppsala, Sweden) followed by evaporation. All samples were reconstituted with 200 ?L methanol (Fisher Scientific, Waltham, MA, USA). The mobile phases used in this method were Millipore water (Synergy UV-R, MilliporeSigma, Burlington, MA, USA) with 0.1% formic acid and 5mM ammonium formate (Fisher Scientific, Waltham, MA, USA) and methanol with 0.1% formic acid.

Results: The LC method was 10 minutes with retention times ranging from 3.5 to 5.7 minutes. For urine and oral fluids analysis, the calibration range for all analytes was established from 1 to 70 ng/mL. The R2 value was > 0.99 for all analytes. Bias and precision were evaluated at 3, 25 and 60 ng/mL, with bias and %CV for within and between run precision had acceptable values within ±20%. The limit of detection (LOD) was 0.1 ng/mL for most fentanyl analogs, with a LOD of 0.01 ng/mL for valeryl fentanyl and furanyl fentanyl. No carryover was detected for any analytes. The percent recovery of all compounds following SLE for both urine and oral fluid was >50%. For urine analysis, the ion enhancement and suppression of all analytes was within 25%. For oral fluid analysis, the ion enhancement and suppression of most analytes was within 25% except valeryl fentanyl, which experienced enhancement of 35%. The matrices analyzed had no effect on the detection or quantitation of analytes in this method. Interference effects of commonly encountered drugs were studied, and showed minimal impacts on the results generated from this method.

Conclusion: Using the QSight™ Triple Quad LC/MS/MS following SLE effectively identified and quantified fentanyl analogs present in both urine and oral fluid. This method has shown its potential to be applied to casework samples for fentanyl analogs quantitation.
Comparison of Sample Preparation Techniques for the Detection and Quantification of Twenty-Three Drugs in Oral Fluid

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Forensic toxicology is a branch of science that involves the analysis of drugs and other substances in biological fluids and tissues such as blood, urine, and oral fluid to aid medical or legal investigation of death, poisoning, and drug use. Due to the various components of different matrices, efficient and effective sample preparation techniques are necessary for reliable and accurate analysis. Following sample clean-up, a sensitive, specific, and robust method is ideal for consistent detection, identification, and quantitation of analytes. With the rise of drug abuse, there is a growing need to develop a single method that can target multiple classes of drugs quickly and effectively.

This study validated two different sample preparation techniques for the detection and quantitation of six drug classes comprised of twenty-three drugs and metabolites in oral fluid. The drug classes were as follows: amphetamines, local anesthetics, opioids, hallucinogens, antidepressants, and novel psychoactive substances (NPS). Amphetamines used were amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethylamphetamine (MDMA), and 3,4-methylenedioxy-N-ethylamphetamine (MDEA). Local anesthetics contained benzoylecgonine (BZE), cocaine, and lidocaine. Opioids included codeine, methadone, morphine, 6-monoacetylmorphine (6-MAM), fentanyl, and oxycodone. Hallucinogens included lysergic acid diethylamide (LSD) and phencyclidine (PCP). Antidepressants were amitriptyline, citalopram, fluoxetine, and trazodone. Lastly, NPS included ethylone, 3-pyrrolidinopentiophenone (3-PVP), and 2,5-dimethoxy-4-iodophenethylamine N-(2-methoxybenzyl) (25I-NBOMe).

Supported liquid extraction (SLE) and solid phase extraction (SPE) were assessed followed by confirmatory analysis by liquid chromatography (LC)-tandem mass spectrometry (MS/MS). Both methods were validated according to guidelines in the Standard Practices for Method Validation in Forensic Toxicology set by the American Academy of Forensic Science (AAFS) Standards Board (ASB). Parameters assessed include calibration model, bias, precision, limit of detection (LOD), limit of quantitation (LOQ), dilution integrity, ion suppression/enhancement, interference studies, and stability. Matrix recovery was added as another parameter. All calibration models were 0.99 or greater and all compounds were stable for at least 72 hours. Bias, precision, LOD, LOQ, dilution integrity, and interferences were similar between both methods. SLE yielded slightly better LOD and LOQ values. SLE had greater values of matrix recovery as well as lower levels of ionization suppression/enhancement.

Overall, SLE was determined to be the better method of sample preparation for this panel of drugs in oral fluid. Not only did it yield higher values for several of the parameters assessed but it also was more efficient (1 hour versus 2 hours) while using less solvent.
Marijuana and Breastfeeding: a Forensic Issue

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Introduction: in France, the number of pediatric cannabis intoxication is increasing since 2014 (ANSM, 2015). These intoxications mainly concern children under 2 years of age and most often result from accidental ingestion of marijuana (ANSM, 2015). However, although direct ingestion corresponds to the most frequent route of administration in children, some cases of pediatric cannabis exposure may represent a real challenge for the toxicologist. Thus, we were asked to answer the following question: are the delta-9-tetrahydrocannabinol (THC) concentrations highlighted in blood of an 18-old-month infant related to direct ingestion of marijuana or related to breastfeeding (regular female user)?

Case history: an 18-month-old infant was tested positive for cannabis. Three consecutive (over 17 hours) blood sample (P) analysis using LC-MS/MS highlighted the presence of THC, 11-hydroxy-tetrahydrocannabinol (11-OH-THC), and 11-nor-carboxy-tetrahydrocannabinol (THC-COOH).

Methods: In order to answer the question, a bibliographic research was performed using several different databases: PubMed, Scopus, Web of Science and Google Scholar.

Results:

P1: THC: 37.5 μg/L, OH-THC: 28.0 μg/L; THC-COOH: 235.0 μg/L;
P2: THC: 44.0 μg/L, OH-THC: 37.2 μg/L, THC-COOH: 403.0 μg/L;
P3: THC: 2.9 μg/L, OH-THC: 6.3 μg/L, THC-COOH: 219.0 μg/L.

Discussion: Children can be exposed to marijuana from different mechanism such as passive inhalation of cannabis smoke or direct ingestion of marijuana. THC can also be found in breast milk where it tends to concentrate due to its lipophilicity. Among mothers who consume marijuana on a regular basis, it is thus possible to find, at variable concentrations, THC, 11-OH-THC, and cannabidiol (but no THC-COOH or cannabino) in the breast milk (Bertrand KA et al. Pediatrics 2018;142 (3); Perez-Reyes M et al. N Engl J Med 1982;307 (103):819-820). Reported THC concentrations can be very high (1-323 μg/L) (Reference center for teratogens, 2018; Drugs and lactation database, 2019) and depend on: i) the time elapsed between the last marijuana consumption and the sampling; ii) the breast milk composition, which present an inter individual variation. Here, the infant THC blood concentration is too high to result only from passive exposure to cannabis smoke, and so, confirms an active exposure (Toennes SW et al. Arch Kriminol 2010;225(3-4):90-8). In addition, 11-OH-THC blood concentrations were similar to THC blood concentrations, which was in favor of an oral administration of cannabis.

Finally, although marijuana direct ingestion remains very likely, the hypothesis that such concentrations were the consequence of breastfeeding cannot be formally excluded. In fact: i) in both cases, the cannabinoids route of administration is oral route; ii) the estimated dose (and bioavailability) of cannabinoids ingested during breastfeeding remains still poorly known. Thus, it appears legitimate to think, in view of these data that the toxicological pattern observed in infants exposed to marijuana via breast milk will be very similar to that observed after direct marijuana ingestion.
Toxicological Analysis of Heroin: Identification of Drug Related Evidence

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Heroin is a semi-synthetic drug synthesized by acetylation of morphine existing in opium poppy tears as a main opiate. Since it is an intravenous illicit drug, heroin addiction is a health concern to all age groups in Europe. Hence, addiction to heroin causes countless illnesses or deaths every year all over the world. According to the Turkey Drug Report (2018): toxicological investigation was reachable for the complete approved drug-associated expiries. More than half of deaths was connected with multi-substances including opioids. In particular, heroin was engaged in nearly one third of the deaths. In previous years, numerous chromatographic techniques were improved to measure heroin levels in seized drugs along with biological specimens for clinical, forensic, toxicological, and pharmaceutical scopes. As an option to immunoassays, thin-layer chromatography (TLC) looks as advantageous regarding to it is one of the most feasible and the economical techniques. Nonetheless, such screening assays are disadvantageous when sample have low concentration of drugs. Consequently, mass spectroscopy (MS) coupled to liquid chromatography (LC) and gas chromatography (GC) are sophisticated instruments for quantification and confirmation. This poster proceeding reviews toxicological analysis of heroin as an identification of drug related substances, involving partial information from corresponding author’s previously published work at Ann Clin Anal Med 2020;11(1):38-42. https://doi.org/ 10.4328/ACAM.6139

A Study of Filter Types Used in Sample Preparation of Cannabis/Hemp with HPLC Analysis

Niloufar Pezeshk, Jonathan Edwardsen and Craig Young, Shimadzu Scientific Instruments, Inc.

Filtration is a critical step in preparing cannabis and hemp samples for HPLC potency analysis. Fine particles must be removed to make the sample suitable for HPLC injection, following extraction into a suitable solvent. Syringe filters, while effective for particulate removal, can sometimes be problematic in terms of analyte adsorption, resulting in some loss of target recovery. We conducted a study to determine the recovery of phytocannabinoids (in terms of concentration) using several syringe filters. Seven types of syringe filters were tested with methanol used as the solvent medium, as per the manufacturer’s recommendation. Our goal was to determine the recovery (without pre-wetting) of phytocannabinoids using seven different types of filters; polyvinylidene difluoride (PVDF-hydrophobic), modified polyvinylidene difluoride (PVDF-hydrophilic), polypropylene (PP), polytetrafluoroethylene (PTFE-hydrophobic), nylon, cellulose acetate (CA) and polyether sulfone (PES). We conducted a modified recovery study using syringe filters for sample preparation with the Cannabis Analyzer for Potency™- an integrated HPLC system with built-in UV detector. We conducted a solvent spiking evaluation of the filters using methanol as the un-spiked, un-filtered solvent. A quantitative HPLC method for the determination of 11 phytocannabinoids was used. Nylon and PTFE syringe filters were the best candidates as they presented minimal hold-up of the phytocannabinoids and stable recoveries among ten replicates.
The Potency Determination of 15 Cannabinoids by Cannabis Analyzer for Potency with UV-Vis Detector
Niloufar Pezeshk and Craig Young, Shimadzu Scientific Instruments, Inc.

Revenue for cannabis testing is set to rise to roughly 2 billion USD by 2025. According to a 2019 report from Global Market Insights, Inc, the chromatography technology segment will account for 1.5 billion USD of this revenue. The potential for market growth is attributed to the ongoing development of chromatography techniques in potency testing of cannabis, supporting manufacturing operations, and the associated clinical science. Since it is available in numerous forms, cannabis is a challenging product to analyze. It consists of hundreds of cannabinoids. This study optimizes a quantitative chromatographic determination of 15 cannabinoids using the Shimadzu Cannabis Analyzer for Potency™.

For this study, a Shimadzu Cannabis Analyzer for Potency™ - an integrated HPLC system with built-in UV detector - was used. We developed a method that builds on the existing High Sensitivity Method, optimized for the quantitative determination of 15 major cannabinoids, in response to the increasing demand for development of chromatography techniques in potency testing of cannabis and hemp. The statistical results document rigorous testing for retention time and peak area repeatability, quantitative accuracy and sensitivity.

Screening for Synthetic Cannabinoids Using Triple Quadrupole and High Sensitivity QTOF Mass Spectrometers
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The number of new designer drugs and their availability has exploded in recent years, leading to widespread social impact on local communities. LC-MS is used to confirm the presence of known substances involved in a particular toxicology case. However, these methods can detect only a few known substances and common designer drugs, but cannot identify new or emerging drugs of concern, like the always-evolving synthetic cannabinoids. We developed a new LC-MS/MS method using a high-sensitivity Q-TOF mass spectrometer and a fast scanning triple quadrupole mass spectrometer combined with an enhanced spectrum library to detect and screen for synthetic cannabinoids to support forensic investigations.

More than 300 authentic standards for emerging synthetic cannabinoids were obtained from Cayman Chemicals (Ann Arbor, MI) and analyzed by a Q-TOF mass spectrometer and a triple quadrupole mass spectrometer (LC-MS/MS) to create both a tandem mass spectrum library and a high-resolution tandem mass spectrum library. In order to create the libraries, product ion spectra were obtained at different fixed collision energies, and for the QTOF, product ion spectra were acquired at a single collision energy of 35 eV with a collision energy spread of ±17 eV. Samples containing unknown compounds were prepared by solid phase extraction and analyzed by LC-MS/MS following centrifugation to remove particulates. Analysis was carried out using UHPLC separation, electrospray ionization, and detection in various MS modes including high resolution scan mode and data-dependent MS-MS.
Potency Determination of 16 Cannabinoids by UHPLC with Diode-Array Detection

Niloufar Pezeshk, Beth Markello and Craig Young, Shimadzu Scientific Instruments, Inc.

With the wide-reaching legalization of cannabis in the U.S., the quantitative determination of cannabinoids in cannabis products is of great commercial interest. There are more than 100 cannabinoids that can be found in the plant or extracts. Tetrahydrocannabinol (THC) and cannabidiol (CBD) are two of the highest priority, in potency testing, along with their acidic forms. The acidic forms, Tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA), are primarily found in the plant, subsequently converting to THC and CBD through decarboxylation from exposure to heat and light. HPLC is the gold standard for cannabinoids analysis, including the acidic forms, providing nearly complete separation of the cannabinoids, and robust quantitation. Several methods have been developed for optimal results of resolution, sensitivity, and throughput. To assist in optimizing for high throughput while maintaining sensitivity and resolution, this study proves a 4.5-minute isocratic method using a Shimadzu Nexera-I (LC-2040C 3D Plus) UHPLC with a photodiode array detector. This method was optimized for the quantitative determination of 16 major cannabinoids in response to the increasing demand for development of chromatography techniques for potency testing of cannabis and hemp. The statistical results show short run times, retention time and peak area repeatability, as well as quantitative accuracy and sensitivity. For each matrix type analyzed, a retention time reproducibility and carryover study should be used to determine the degree of matrix effects over time between injections, and what type of column washing should be implemented.

Fully automated detection of phosphatidylethanol (PEth) from dried blood spots (DBS)

Oliver Lerch, Ph.D., GERSTEL GmbH & Co. KG

An automated determination of the novel alcohol consumption markers phosphatidylethanol (PEth 16:0/18:1, PEth 16:0/18:2) in dried blood spots (DBS) has been developed. Blood samples were taken by HemaXis DB 10 device and simply positioned on an MPS autosampler for analysis (LOQs <10 ng/mL).
Multi-Target Screening of Toxicological Compounds in Blood on a Fully-Automated LC-MS/MS Platform

Nat Tansrisawad¹, Udomsak Hoonwijit¹, Apinya Tubtimrattana², Boontariga Intawong¹, Samita Tanasarnsopaporn², Jakkapan Boonsritan¹, Zhe Sun³, Chukkapong Comsup³, Prapath Tienprateep⁴, Zhaoqi Zhan³
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Multi-target screening by LC/MS/MS has been widely adopted in detection and quantitation of drugs of abuse (DoA) in forensic investigation and toxicological research. Usually, a wide range of targets are screened in such analysis, including illicit drugs, narcotics, psychotropics, antipsychotics, pharmaceuticals and other toxic compounds in urine, serum/plasma and whole blood samples. Sample preparation is often a bottleneck due to the tedious steps. It is also a factor responsible for inaccurate or false negative results. We describe a solution by using an automated sample preparation module CLAM 2000TM connected with LC/MS/MS system (LCMS-8060) for multi-target screening of 61 drugs in whole blood. A ready-to-use method package Rapid Toxicology Screening (Shimadzu) was used to set up the screening method with human whole blood (frozen) spiked sample without efforts in LC and MRM method development.

A study on the Extraction of Mitragynine by Using Solid Phase Extraction

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Kratom is a plant-based novel psychoactive substance that has gained popularity in recent decades in United States as a recreational drug of abuse. It is indigenous to South East Asia where people use it in traditional medicine and recreationally as a stimulating drug. Although, kratom is not a scheduled drug in the United States, it is in the list of drugs of concern of the FDA due to its potential for abuse. Mitragynine is one of the main active alkaloids in the plant Mitragyna Speciosa Korth (Kratom). The consumption of products from the herbal plant in low doses has stimulating effects while at higher doses sedative opioid-like effects are observed. The presence of mitragynine in biological fluids is used as a biomarker for the detection of Kratom abuse in humans.

The research presented here is concerned with the solid phase extraction of mitragynine from body fluids, specifically renal fluid. Three different commercially available solid phase extraction columns were tested. Analyte recovery rates and purity of eluates were compared. The goal of this investigation was to develop a robust, highly reproducible method that produces samples suitable for GCMS and LCMS analysis in the field of forensic toxicology.
Barbiturates are central nervous system depressants belonging to the sedative-hypnotic classification. Suicide by barbiturates overdose has been reported in the scientific literature since the 1950s. We examined the 31 cases of suicide by barbiturates overdose recorded in the file archive of the forensic/medicolegal section of the university hospital of Parma, Italy, between 1954 and 2014. The total number of cases was 31 in a population increasing from 390,000 to 450,000 in sixty years (1954-2014). The peak number of cases was between 1964 and 1976. The male/female ratio was 1.58/1. The median age at death was 37.6 years. Based on external examinations and autopsy reports, as well as case histories and newspapers articles, all 31 deaths were ruled as suicides. The analytical techniques on biological matrixes gradually developed from immunoassays to GC/MS, through Thin-Layer GC. Literature data were confirmed with regards to the lethal dose. Results were mostly consistent with the extensive literature about suicide by barbiturates overdose, which has become rarer and rarer in the last 35 years. Surprisingly, male deceased were more numerous than female, and an explanation was proposed, as well as the hypothesis about the reason why no barbiturates with ultra-short duration of action were involved. In a socio-cultural context, we noticed how evidence in the past relied much more on case histories than on science, that is police reports and newspapers articles vs. toxicology. Similarly to the evolution of requirements connected to evidence, toxicology has evolved, both through the technology of analytical methods and in the procedural guidelines, such as the current necessity of two distinct techniques, based on different analytical principles, for screening and confirmation (e.g. EMIT and GC/MS). Finally, before the decline of medical prescriptions 35 years ago, aspiring suicides acquired barbiturates by prescription or by seizing them in sanitary institutions, such as hospitals or emergency rooms. Dissimilarly, the 2000s witnessed the rise of the illegal market, either physical or, more commonly, online through the dark web.
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