Annual Conference 2018

1st and 2nd of November

Hilton Hotel Kilmainham, Dublin

Welcome to Dublin for UKIAFT 2018

The organising committee would like to extend you a warm welcome to Dublin, for the annual UKIAFT Conference. The organisation of this year's meeting was achieved through collaboration between the Medical Bureau of Road Safety, the Irish State Laboratory and the HSE National Drug Treatment Centre. We hope that you have a great experience during your visit!

Dublin City

Dublin is Irelands capital city with a population of over 1 million people. Dublin is in the province of Leinster and is situated on the east coast at the mouth of the river Liffey. Dublin is bordered on the south by the Wicklow mountains which provide a great escape for Hill walkers. This city has many museums and sites of historic interest as well good shopping, restaurants and a vibrant nightlife. It is well situated for exploring anywhere in Ireland.

Hilton Kilmainham

Situated in a prime Dublin location, the Hilton Dublin hotel is close to some of the city's top visitor attractions including the Irish Museum of Modern Art, the historic Kilmainham Gaol Museum and the Phoenix Park.

Guinness Storehouse

Located in the heart of the St. James's Gate Brewery, the Guinness Storehouse is Ireland's most popular tourist attraction. The journey begins at the bottom of the world's largest pint glass and continues up through seven floors filled with interactive experiences that fuse a long brewing heritage with Irelands rich history. At the top, you'll be rewarded with a pint in the world famous rooftop Gravity Bar. Delegates will be able to explore the storehouse and later in the evening dinner will be served.

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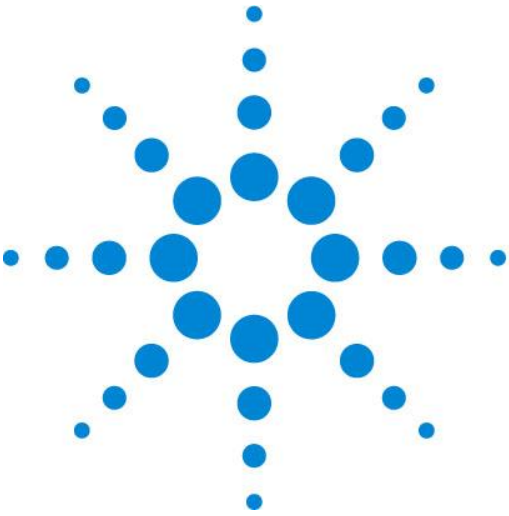

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Organising Committee

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Yvonne Kavanagh	State Laboratory
Helen Kearns	MBRS
Richard Maguire	MBRS
Peter Streete	UKIAFT Treasurer/Website Liaison

Special thanks to all of the following who helped to organise this year's meeting

Karen Hayes

Clare Morgan

Monica Whelan

Sinead McNamara

Carol Gleeson

Veronica Gubarkova

Tony O'Hara

John Bradley

Aisling Kennedy

Olivia Johnston

Meeting Program

Thursday 1st November

10:30 - 13:00 Registration & Information Desk

09:30 - 12:30 UKIAFT Business Meeting – Members only

12:30 - 13:45 Lunch and Poster session with exhibitors

Scientific Session 1			
Time	Mins	Title	Speaker
13:45 - 14:00	15	Welcome	Y. Kavanagh, S. Patterson
14:00 - 15:00	60	Plenary 1: Opioid Epidemic.	C. Walls
15:00 - 15:30	30	Coffee Break	n/a
15:30 – 16:00	30	Agilent: Fast LC-HRMS Screening by Q-TOF in Doping Control.	B. Wüst
16:00 – 16:30	30	The evidence base for suggested changes to UKIAFT ATD guidelines.	M. Scott Ham & P. Maskell

Friday 2nd November

Scientific Session 2			
Time	Mins	Title	Speaker
09:30 - 10:30	60	Plenary 2: Forensic toxicology - an Irish Pathologist's perspective.	L. Mulligan
10:30 - 11:00	30	The National Drug-Related Deaths Index: Using toxicology results to highlight public health issues.	E. Lynn
11:00 - 11:30	30	Coffee Break	n/a
11:30 - 12:00	30	Chemsex: A Deadly Addiction & the GHB Detoxification Clinic.	K. Santlal
12:00 - 13:00	60	Hair analysis: In theory and in practice.	R. Kronstrand
13:00 - 14:00	60	Lunch	n/a
14:00 - 14:30	30	Implications of the Introduction of Section 5A Road Traffic Act Offence in Scotland	J. Officer
14:30 - 15:00	30	Specialty Diagnostix: Pregabalin, Gabapentin and Fentanyls: A brief history and an update.	K. Williams
15:00 - 15:30	30	Enforcing Irish Road Traffic Law.	P. Woods
15:30 - 15:45	15	Coffee Break	
15:45 - 16:00	15	Vendor Info	S. Stokes
16:00 - 16:30	30	DUID in Ireland Update.	R. Maguire
16:30 - 16:40	10	Meeting Close	Y. Kavanagh, S. Patterson

Invited Speakers

H. Chip Walls

Opioid Epidemic.

The evolving face of the opioid overdose epidemic from heroin to prescription drugs back to heroin plus synthetic opioids--an epidemic within an epidemic; Use and abuse of benzodiazepines with Opioids, the underbelly of the overdose epidemic. A *syndemic!!!*

There are many misconceptions about the true number of fatalities that opioids are responsible for- "How are we counting the bodies?". Have deaths from traditional drugs of abuse declined or disappeared? Does despair + drug availability + overdose and/or polydrug ingestion lead to deaths ruled as suicide or accidental death? And then there are synthetic opioids + synthetic benzodiazepines --the perfect storm What is the chemistry of benzodiazepines and opiates? What is the pharmacology of co-ingestion of benzodiazepines and opiates that leads to such synergistic toxicity? Why are so many people prescribed or choosing to use benzodiazepines + opioids? Is this exacerbated by doctor shopping in the U.S.? Is Narcan the mop? This presentation will outline the history and toxicology of opioid use to answer the question: "How did we get here?"

Chips professional career has covered more than 44 years. His experience encompasses post-mortem forensic toxicology, clinical toxicology, and probation urine drug testing, and driving under the influence cases. Currently, he owns and operates the Forensic Analytical & Clinical Toxicology Laboratories Consulting & Training Specialists in Miami.

He has served the Society of Forensic Toxicologist (S.O.F.T.) as President (1996). 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 2017, he was recognized by the National Highway Safety Administration 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.

Dr. Linda Mulligan

Forensic toxicology – an Irish Pathologist's perspective.

Dr. Linda Mulligan is a medical graduate of UCD. Following three years in clinical medicine in Ireland and Australia, she completed her Histopathology training in Dublin. She obtained her Fellowship of the Royal College of Pathologists in 2013 and went on to train as a Forensic Pathologist at the State Pathologist's Office (OSP). She currently works as one of the Deputy State Pathologists in Ireland.

The presentation will cover current practices and interactions between hospital and forensic pathologists in Ireland with the State Laboratory. It will highlight the difficulties faced in toxicology interpretation, from a pathology point of view, using cases as examples. It will also include some recent toxicology-related research carried out by the Office of the State Pathologist.

Dr. Robert Kronstrand

Hair analysis: In theory and in practice.

The analysis of drugs in hair has been routine for over three decades to determine drug use and abuse in the past in both the dead and the living. More recently the high sensitivity of new methodologies have also made it an important addtional matrix in cases of suspected drug-facilitated crimes when a single intake of a drug is to be detected. Therefore, it is important to understand the limitations of hair as a matrix as well as all the issues surrounding the sampling, analysis and interpretation. Professor Kronstrand will give his view of the important factors that should be taken into account when performing analysis and interpreting results.

Professor Robert Kronstrand has close to 30 years of experience in forensic toxicology. In addition to numerous expert opinions in the field of ante- and post-mortem forensic toxicology, Professor Kronstrand has engaged in research including opiate toxicology, pharmacokinetics, pharmacodynamics, and, not least, in research involving the analysis of drugs in hair. After pursuing his doctoral degree in hair analysis he continued to investigate the incorporation, detection and interpretaion of hair analysis and is the author of 26 publications related to hair. Professor Kronstrand is also the vice President of the Society of Hair Testing.

Ms. Ena Lynn

The National Drug-Related Deaths Index: Using toxicology results to highlight public health issues.

This presentation will illustrate the National Drug-Related Deaths Index's (NDRDI) methodology of data collection from the Coronial Service. The majority of the presentation will have specific emphasis on the importance and usage of toxicology results in relation to highlighting public health and safety issues and in influencing policy and practices in these areas. Examples presented will include the areas of drug-related deaths, epilepsy, fire fatalities and road traffic collision fatalities.

Ena Lynn is a Research Officer at the Health Research Board. Her role is to oversee the day-to-day management of the National Drug-Related Deaths Index (NDRDI). She graduated as a general nurse and midwife and after eight years' experience in the hospital environment, moved into the research sphere. Ena completed a Masters in Health Promotion with a specific interest in evidence based practises. Ena helped set up and implement the NDRDI and has been instrumental in its expansion into other public health areas. She also completed a course in medical toxicology. Ena has a passion for usability and information sharing. She has recently commenced a PhD in conjunction with the RCSI.

Dr. Kiran Santlal

Chemsex: A Deadly Addiction & the GHB Detoxification Clinic.

Gamma hydroxybutyrate is increasingly becoming a substance of misuse, and of dependence. Its illicit use has been in the context of clubs, after-parties and chemsex parties, in conjunction with a number of other illicit substances. In Ireland, it is currently the most commonly used drug for chemsex. Chemsex refers to any sexual activity whereby participants use drugs specifically to enhance and facilitate the sexual experience. The National Drug Treatment Centre, since 2014, has had a number of referrals for admission onto the Detoxification Programme. These referrals have been facilitated and the detoxifications have been successful. In this presentation, Dr. Santlal will present a number of cases identified, and discuss the short and long-term strategies that would be recommended to decrease the risk of a relapse.

Dr. Santlal has worked in the HSE-National Drug Treatment Centre, since 2015, as a Registrar in Psychiatry in the Methadone Maintenance & Stabilisation Programmes. He has a special interest in Gamma Hydroxybutyrate and developed The GHB Detoxification Clinic, under the supervision of Dr. Eamon Keenan, HSE-National Clinical Lead in Addictions & Dr. Fiona Fenton, Consultant Psychiatrist in Substance Misuse and with testing by the Laboratory of the HSE-National Drug Treatment Centre. He has presented the GHB Detoxification Clinic and its related research to various Irish Hospital Medical and Psychiatric departments. He has also presented it at the Club Drug Health Conference, Gay Health Forum, Drugs & Alcohol Task Force and the Probation Service, as well as the Royal College of Psychiatrists International Conference and College of Psychiatrists in Ireland Conference.

Sponsor Speakers

Agilent

Mr. Bernhard Wüst

Global Marketing Manager, Sports Doping / Forensics at Agilent Technologies, Germany

Fast LC-HRMS Screening by Q-TOF in Doping Control

Since some years high resolution accurate mass screening has made its way into doping control labs. This presentation describes how analytical challenges are dealt with using an Agilent 1290/6545 LC-QTOF instrument acquiring in positive and negative mode simultaneously. In method one the lab is required since 2016 to analyse all samples qualitatively for meldonium and semi-quantitatively for ethyl-glucuronide. In method two the labs standard screening method has been adopted to the QTOF instrument. Approximately 250 compounds were analysed by the LC-QTOF using fast pos/neg switching. Data review was readily achieved using the Qualitative and Quantitative Mass Hunter software, and its strengths and weaknesses to doping control applications will be discussed.

Mr. Bernhard Wüst completed his analytical chemistry engineer studies at a German food and veterinary institute. After joining Hewlett Packard in 1992 as customer engineer he joined the field marketing department as an application chemist. Being part of factory advisory teams for various products on GC/MS and LC/MS products he worked in a team to develop Agilent's accurate mass instruments. He left Agilent in 2004 to work in South Africa as a product specialist in sales. Returning to Agilent Technologies, Germany in 2009, he joined the R&D and marketing department in Santa Clara, California in 2010 with the global responsibility for Chemical Analysis Applications which includes food, environmental and forensic/toxicology applications. He pioneered various software developments for high resolution data and applications including accurate mass libraries as well as been part of hardware developments and improvements. In 2014 he took over the responsibility as a Global Marketing Manager for Sports Doping / Forensics. In this role, he collaborates with anti-doping labs, human and animal doping. He also supports the European forensic markets, mainly in high resolution accurate mass spectrometry applications in understanding requirements and developing and improving products and software.

Specialty Diagnostix

Dr. Keith Williams

Pregabalin, Gabapentin, and Fentanyls. A brief history and an update.

Pregabalin and Gabapentin: Pregabalin and gabapentin are two close related substances independently created to aid in the treatment of epilepsy and potentially other conditions. Their development as drugs, changing patterns of use and changing legislation will be covered, along with presentation of recently released data from government sources and recent conferences. An overview of detection methodologies and reference materials is also included. Fentanyl(s): Fentanyls are

continuing to attract attention in the press, politically and in testing laboratories throughout the world. The combination of their relative ease of manufacture and in some cases, extreme potency and potentially high profit margins though illicit dealing provides a ready end user market. One of the challenges is consistent nomenclature to ensure that there is a common language involved. Detection of such materials can be challenging and may require sophisticated analytical equipment. A brief overview of the current state of fentanyls will be given, including an overview of detection methodologies and reference materials.

Dr Keith Williams has been involved in forensic toxicology and determination of xenobiotics in biofluids. Initially, his work was based at the University of Glasgow, researching the detection and effects of Anabolic Agents in the Racing Greyhound before moving to the University of Dundee in 1993. There he delivered the Forensic Toxicology service for part of Scotland, as well as undertaking teaching and research prior to moving to London in 1997. In London, Keith managed laboratory operations and reported coroners and criminal forensic cases (from drink/drug driving to serious crime cases at the Old Bailey) as well as running a large work place drug testing laboratory while drifting into animal regulatory toxicology for a brief time. More recently he has been involved in the quality aspects of laboratory operations, particularly surrounding the production and use of reference materials, covering not just Forensic Science but also the pharma, environmental and industrial market spaces. Highlights include appearing at almost all levels of the court systems in England, Scotland, Wales with the occasional appearance in Northern Ireland, presenting some of the earliest use of hair analysis in court in a case of non-self induced automatism, and watching in fascination as a particular appeal case went horribly wrong for the defendant.

Oral Presentations

O1: The evidence base for suggested changes to UKIAFT Alcohol Technical Defence (ATD) guidelines

Peter D Maskell[a*], Michael Scott-Ham[b]

[a]Abertay University. Dundee. Scotland. UK. [b]Principal Forensic Services, Bromley. Kent. UK

In 2014 UKIAFT agreed the latest version of the Alcohol Technical Defence Document, this provides a set of guidelines for performing blood alcohol calculations when used in forensic casework. This document was based on the most up-to-date evidence that was available at the time. Since the publication of version 2.1 of the Alcohol Technical Defence Document more evidence has been published that should be considered for inclusion.

This includes the postulation of the question. How certain can we be that the declared % alcohol by volume on an alcoholic beverage package is accurate?

Should we be carrying out ATD calculations when only estimated values for weight are given?

This includes which is the most reliable anthropometric method to calculate the volume of distribution (V_d) of ethanol in an individual?

Recent data has suggested that highly aromatic beers, particularly popular with 'craft beer' consumers, (an example being IPA's, which may even be 'double' or 'triple' hopped) exhibit the largest variation in actual vs declared % ABV. The variation for "Big" beer was limited by comparison.

Furthermore, when considering the mass of an individual, there can be up to a 15% variation in true weight when compared to weight when estimated by an observer.

Uncertainty in total body water has been raised as an issue to be considered when performing certain alcohol-related calculations. This has been reviewed by undertaking an evidence-based survey into the reliability of various anthropometric based methods for the determination of the V_d of alcohol. This showed that the Watson, Watson & Batt and Forrest Anthropometric equations are the most reliable. This study also determined 95% confidence intervals for the individual anthropometric calculations.

In the presentation we also show how these recent studies can be used, changing and improving ATD calculations.

O2: Implications of the Introduction of Section 5A Road Traffic Act Offence in Scotland

Jane Officer, Scottish Police Authority

Introduction: It is proposed that from October 2019, Section 5A of the Road Traffic Act 1988 will be introduced in Scotland. It will become an offence to drive while the concentration of any specified drug in blood is above a limit set for that drug. The proposed specified drugs and their limits will remain the same as currently in place in England and Wales. It is necessary to estimate the number cases in Scotland where the concentrations of drugs found will be above the proposed common reporting threshold (CRT) for Section 5A. Secondly, it is important to determine how many cases would likely not be prosecuted under Section 5A either because a drug concentration is under the CRT or the drug(s) present are not listed in Section 5A.

Method: Results from samples submitted to Scottish Police Authority (SPA) under the current Section 4 offence during July to December 2017 were collated. The concentrations of the 17 specified drugs were compared against the proposed CRT to determine the likely proportion of cases that will be prosecuted under Section 5A when the offence is introduced. The drugs found that are not included in the Section 5A offence will also be assessed and the proportion of cases that will likely be prosecuted under the Section 4 offence determined.

Results: 261 cases were submitted to SPA under the current Section 4 legislation during July to December 2017. The most commonly detected drugs were Cannabis (51%), Diazepam (49%), Morphine (38%), Methadone (30%), Cocaine / BZE (28%), Etizolam (28%) and Alprazolam (27%). Where concentrations were available, 14% of Diazepam cases were above the CRT set for Section 5A. Likewise, 30% of cannabis (THC); 5% of Morphine; 0% for Methadone; 100% for Cocaine; and 93% for BZE. Amphetamine, MDMA and Temazepam are also listed in Section 5A legislation and 24%, 84% and 0% of results respectively had concentrations above the CRT. The remaining drugs in Section 5A were either not detected in any cases (Flunitrazepam, LSD); not routinely quantified by SPA (Oxazepam, 6-Monoacetylmorphine); or detected in only a very small number of cases (Clonazepam (2), Ketamine (4), Methylamphetamine (3)). Lorazepam is also found as a metabolite of Diclazepam and only one case was positive for Lorazepam in the absence of either Diclazepam or other Diclazepam metabolites and all cases of Lorazepam were below the CRT. In total, 57% of Section 4 blood samples were not positive for any Section 5A drug above the CRT.

Conclusion: From 2017 drug driving data, it is expected that a significant number of Section 5A cases in Scotland will either be negative for the specified drugs or fall below the Common Reporting Threshold. It will be necessary for Police to engage a Forensic Medical Practitioner to declare drivers are impaired through drink or drugs to allow prosecution of these cases using the current Section 4 legislation.

O3: DUID in Ireland: An Update

Richard Maguire

Medical Bureau of Road Safety, University College Dublin

Introduction: The 2016 Road Traffic Act in Ireland introduced several measures to tackle driving under the influence of drugs, including the ability for An Garda Síochána (AGS) to conduct roadside and station-based drug testing using oral fluid. At the same time *per se* drug levels were introduced for Cocaine, Cannabis and Heroin. A medical defence for patients on prescription cannabinoid based medicines containing Δ^9-Tetrahydrocannabinol was included. Where an individual has a prescription for an approved medicine the *per se* law does not apply and impairment must be proven. This update reviewed the available data from April 2017 to date including enforcement activity and laboratory findings.

Method: Data from the roadside and station-based testing as well as laboratory screening and confirmation testing was reviewed to evaluate the newly introduced measures. Roadside and station-based testing is conducted using the Drager DT5000. This system is used to detect Cannabis, Cocaine, Opiates and Benzodiazepines. The laboratory screening is conducted using immunoassay for Cannabis, Cocaine, Opiates, Benzodiazepines, Amphetamines, Methamphetamines and Methadone. Laboratory confirmation is conducted using GC-MS-MS and LC-MS-MS testing on whole blood. In addition, data available from AGS was also considered.

Results: Before the enactment of the new measures the driver had the option to provide either blood or urine. When a specimen of oral fluid is collected and found to be positive for cannabis, cocaine or opiates the driver is compelled to provide a blood specimen. Since the introduction of the new measures there has been a marked increase in the number of blood specimens collected relative to urine. The Drager DT5000 is working well and has performed satisfactorily. There is good agreement between the oral fluid testing system and the laboratory findings. Cannabis and Cocaine are the most prevalent drugs detected using the oral fluid testing system. Opiate and benzodiazepine prevalence are lower. Since the introduction of per se levels 863 certificates of analysis have been issued where a drug/metabolite was found to be in excess of the *per se* level. Most cases are in excess of the *per se* levels stated in legislation for Cannabis and/or Cocaine. There has been only one case where 6-AM was in excess of the *per se* level.

Conclusion: The new measures are working well; however ongoing review is required in order to fully evaluate the impact of the legislative changes.

Posters

P1: Evaluation of Matrix Component Removal Using a Novel Flow-Through Scavenging Plate for Drugs of Abuse Testing in Urine

Rhys Jones, Adam Senior, Helen Lodder, Lee Williams, Geoff Davies, Katie-Jo Teehan, Alan Edgington, Steve Jordan, Claire Desbrow, Paul Roberts

Biotage GB Limited, Distribution Way, Dyffryn Business Park, Hengoed, CF82 7TS, UK.

Introduction: Dilute and shoot (D/S) is the most common form of sample preparation for the analysis of drugs of abuse (DoA) in urine. However this technique presents issues resulting in increased MS downtime. This poster evaluates the extraction of a range of DoA from hydrolysed and non-hydrolysed urine using ISOLUTE HYDRO DME+. Specific matrix-component removal will be demonstrated for creatinine, urea, salt residue, protein and pigmentation associated with urobillin.

Method: LC-MS/MS analysis was performed using a Waters ACQUITY UPLC coupled to a Quattro Premier XE triple quadrupole mass spectrometer. A DoA panel was chromatographed using a Restek Raptor Biphenyl HPLC column, while alternative chromatography for creatinine and urea was provided using a Thermo Betamax acids column. Protein removal from hydrolysed urine was investigated via GE (gel electrophoresis).

Results: D/S was performed using 100 µL of hydrolysed or non-hydrolysed human urine diluted to 1 mL with aqueous buffers. Scavenging performance was evaluated using 100-250 µL of hydrolysed or non-hydrolysed urine mixed with 600 µL of ACN followed by filtering through the scavenging plate or column. Effective removal of salt and pigment components were visually demonstrated following evaporation. Creatinine and urea removal typically demonstrated removal of 90% of both analytes.β-glucuronidase enzyme adds substantial protein content to the matrix, typically between 60-220 kDa. Although dilution lessens this content subtle bands were still visible in GE testing. The scavenging plate completely removed all protein when using 100-25 µL of hydrolysed urine.Full scan and post column infusion experiments showed substantial matrix clean up compared to D/S.Analyte recoveries are typically 50-90% with corresponding RSDs <10% and overall signal was enough to reach cutoff levels for the DoA classes due to lower suppression. Calibration curves (1-400 ng/mL) demonstrated good linearity with r^2 values >0.99 for all analytes. Following direct injection (1 µL) of the eluate most analytes demonstrated LOQs at low or sub ng/mL levels.

Conclusion: This poster demonstrates the use of a novel approach for the extraction and cleanup of a range of DoA from hydrolysed and non-hydrolysed urine. The technique provides excellent removal of many interfering components associated with urine testing resulting in less frequent instrument cleaning, column replacement and associated downtime compared to dilute and shoot approaches.

P2: Evaluation of Simplified Workflow for Hair Matrix Extraction Prior to LC-MS/MS Analysis.

Lee Williams, Katie-Jo Teehan, Rhys Jones, Adam Senior, Helen Lodder, Geoff Davies, Alan Edgington, Steve Jordan, Claire Desbrow, and Paul Roberts

Biotage GB Limited, Distribution Way, Dyffryn Business Park, Hengoed, CF82 7TS, UK.

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

Method: Hair samples 10-20 mg were subjected to micro-pulverized extraction in methanol using the Lysera bead disruption system. Methanolic extracts were cleaned-up using supported liquid extraction, ISOLUTE® SLE+ in 400 µL capacity 96-well plates or columns. Manual positive pressure processing was compared to the Extrahera automated sample preparation platform. Extracts were evaporated at 40 °C and reconstituted in mobile phase. LC-MS/MS analysis was performed using a Waters ACQUITY UPLC coupled to a Quattro Premier XE triple quadrupole mass spectrometer.

Results: A panel of widely abused drugs was investigated comprising of: amphetamine type, opiates, benzodiazepines, cocaines, fentanyls and buprenorphines. Hair extraction was investigated to determine maximum matrix/solvent proportions. Methanol with or without pH modification was chosen for the extraction solvent due to hair swelling ability allowing effective analyte release from the matrix. Sample clean-up was performed using supported liquid extraction. In order to reach SoHT LoQs we compared methanolic extract evaporation and concentration with minimal dilution with aqueous solvent and finally direct extraction of the methanol extract. Typical recoveries were > 80%, RSDs < 10%, good matrix factors and overall signal response. A range of extraction solvents were applicable depending on the exact panel required. Calibration curves (10-1000 pg/mL) demonstrated good linearity with r^2 values >0.99 for all analytes. LoQs were below required SoHT quidelines for analyte panels for both screening and confirmation. Extrahera processing allowed an automated extraction procedure directly comparable to offline positive pressure processing. Full details of optimized workflow will be demonstrated.

Conclusion: This poster demonstrates a simplified workflow for the extraction and cleanup of a range of DoA from hair matrix.

P3: Confirmatory LCMS/MS analysis of more than 100 widely prescribed and abused drugs on the Irish market (60 quantitatively and 43 qualitatively)

Eleanor Dixon, Yvonne Kavanagh, Mark Cronly, Sinead Dunlop, Carol Gleeson & Julie Tierney

State laboratory, Youngs Cross, Celbridge, County Kildare, Ireland

Introduction: A constant challenge in post-mortem toxicology is the balance between satisfying the clients' demand for a sufficiently complex and timely service whilst ensuring the scope of testing adequately reflects the current drug market. In order to meet this demand, we have developed a method for the simultaneous quantitation of 60 drugs and qualitative identification of 43 drugs in post-mortem whole blood.

Method: The method uses a simple liquid-liquid extraction procedure to extract basic and neutral drugs including benzodiazepines, tranquillisers, tricyclic antidepressants, antidepressants, SSRIs (selective serotonin reuptake inhibitors), hypnotics, antipsychotics, anxiolytics, sedatives, analgesics, anticonvulsants, antiepileptics, antihistamines, anaesthetics, antiarrhythmics, antihypertensives, antiemetics, narcotic analgesics, narcotic stimulants and novel psychoactive substances. The extracts are analysed using an ABSciex 4500 Qtrap in positive electrospray mode using MRM transitions.

Results: The method is in routine use in our laboratory with an average throughput of 6000 toxicology samples per year. Real case samples range from low therapeutic to lethal overdose levels. This presents the challenge of analysing and quantifying such a mix of drugs at the levels of interest, within the limits of the dynamic range of the instrument. We describe our approach to overcoming this challenge by either de-tuning or quantifying using ^{13}C isotopes depending on the analyte. We have developed a highly efficient & comprehensive toxicological quantitative method which has been validated according to UKIAFT guidelines.

Conclusion: This method has been instrumental in improving efficiencies both within the section (e.g. replacing individual methods for single drug classes) and has been pivotal in optimising the service provided to the Irish Coroners Service.

P4: Confirmatory LCMS/MS analysis of 19 acidic drugs and metabolites

Carol Gleeson, Yvonne Kavanagh, Eleanor Dixon & Julie Tierney

Human Toxicology Section, The State Laboratory, Backweston Laboratory Campus, Celbridge, County Kildare, Ireland.

Introduction: There is a diverse range of drugs such as barbiturates, anticonvulsants and NSAIDs which are acidic in nature. They are an important category in post-mortem toxicology due to the potential for abuse (e.g. Pregabalin), the potential for accidental or intentional lethal overdose and due to the critical importance of therapeutic compliance. Due to their chemical properties, they typically require a separate extraction procedure to other drugs. An added complexity of analysing them in a single method is their varied range of therapeutic and toxic concentrations (e.g. diclofenac - therapeutic 0.05-2 ug/ml and valproic acid - therapeutic from 50-150 ug/ml).

Method: We present here a simple and efficient protein precipitation method for the confirmatory analysis of 19 different acidic drugs and their metabolites in post mortem blood. The method includes pregabalin, a variety of NSAIDS, barbiturates, anti-epileptics and a cocaine metabolite. The extracts are analysed on an ABSciex 4500 Qtrap with positive and negative electrospray ionisation and scheduled MRM.

Results: The method is in routine use in our laboratory which analyses an average of 6000 toxicology samples per year. It has been validated according to UNDOC guidelines.

Conclusion: The method compliments our general basic/neutral drug method and is a critical part of the toxicology service we provide to the Irish Coroners service.

P5: Post mortem drug screening by High Resolution Liquid Chromatography-Mass Spectrometry

Mairéad Webster, Paula Allen, Eleanor Dixon and Yvonne Kavanagh

Human Toxicology Section, The State Laboratory, Backweston Laboratory Campus, Celbridge, County Kildare, Ireland.

Introduction: Reliable, sensitive and comprehensive drug screening is of critical importance in toxicology and can be achieved via immunoassays and/or gas or liquid chromatography-mass spectroscopy (GC/LC-MS) techniques. Traditionally immunoassay has been considered the gold standard for screening but with the introduction of more affordable high resolution HR/LC-MS instrumentation there is now increased interest in its applications. HR/LC-MS offers a number of advantages including no requirement to optimise fragmentation of analytes of interest in multidrug screens and the possibility of retrospective analysis without re-extraction of the original sample. HR/LC-MS techniques are now complementing or replacing the methodology in toxicology analysis in both clinical and forensic laboratories. Herein we present our current HR/LC-MS methodology employed in post mortem drug screening at the Irish State Laboratory. Our screening method is currently the first phase of testing with confirmatory analysis assigned based on the initial screen results.

Methods: Analysis is performed using a Thermofisher QExactive Orbitrap HR/LC-MS with positive and negative mode switching. The samples screened are blood, urine and vitreous. Blood samples are prepared by protein precipitation while urine and vitreous samples are diluted and filtered. Presumptive positives are identified by matching their exact mass and retention times with those found in our in-house database.

Results: This qualitative screen allows for the detection of up to 167 analytes encompassing benzodiazepines, tranquillisers, tricyclic antidepressants, antidepressants, SSRIs (selective serotonin reuptake inhibitors), hypnotics, antipsychotics, anxiolytics, sedatives, narcotic analgesics, narcotic stimulants, analgesics, anticonvulsants, antiepileptics, antihistamines, anaesthetics, antiarrhythmics, antihypertensives, antiemetics and novel psychoactive substances (which include cathinones, synthetic cannabinoids, synthetic opiates, aminoindanes etc.) in addition to a number of relevant metabolites.

Conclusion: This key analytical methodology is pivotal in providing an improved and optimised screening service for the Department of Justice, (Coroners Service and Criminal Investigations) and can also identify previously “invisible” drugs.

P6: Post-mortem redistribution and the bladder

Emma Lomas, PhD MSci (West Yorkshire Joint Services and University of Huddersfield) and Peter Maskell, PhD, BSc (University of Abertay)

Introduction: There is a reoccurring reason in forensic toxicology to describe the unreliability of post mortem drug levels and it refers to post mortem redistribution (PMR). However, there has been limited research into how and why this occurs.

Objective: This research was based around the investigation of post mortem redistribution from the bladder as this was raised from a previous case study in Japan however; it has not been investigated further. The process was investigated by determining the length of time it takes to breakdown after death and then from this determine how long it would take drugs to be able to diffuse from the bladder.

Method: The diffusion studies involved diffusing rhodamine B and two antidepressants (amitriptyline and nortriptyline) through porcine bladders to determine the intra and inter-variability through the bladder over a period of 5 days post mortem. Franz cells were used to compare physiological and post mortem temperature, pH and tissue degradation to determine the effect of these parameters on the bladder tissue. In addition, whole bladders were included in the research for more realistic conditions of the bladder that would be found in real casework. These factors assisted in determining the effect on the degradation of the bladder and therefore the amount of diffusion, determined by the drug concentration, through the bladder after death.

Results: The temperature experiments resulted in higher diffusion of the control substance and the two antidepressants at physiological temperature(37°C) with peak concentrations at 3.46 ± 2.72 mg/L (intra-bladder, pH 5) for rhodamine B, 6.69 mg/L (amitriptyline) and 6.69 ± 4.76 mg/L (nortriptyline) both at the physiological pH 7.4. However, this was the only parameter to show statistical significance, the other parameters including tissue degradation, solution pH for the bladder sections and the initial drug concentration and bladder volume for the whole bladders showed no significant differences between the physiological and post mortem values. Comparing these parameters the peak concentration of the control substance (rhodamine B) was 3.5 ± 1.02 mg/L at pH 7.4 and for the two antidepressants were 6.68 mg/L (amitriptyline) and 6.69 ± 4.76 mg/L (nortriptyline).

Conclusion: In conclusion, this research determined the bladder is not a source of post-mortem redistribution up to and including 100 hours after death. This was due to the low concentrations of drugs that were released from the bladders over this period, with the largest concentration at 6.69 mg/L.

P7: Clinical toxicology testing application by LC/MS

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Introduction: Structural variants of fentanyl ("designer fentanyls") present particular challenges to the analytical chemist. The high potency of these opioids means that there are usually only very low levels of material may be found in biological samples, so that they may be overlooked, particularly if other 'traditional' opiates are present. Their emergence represents the greatest challenge to forensic toxicology since the appearance of the synthetic cannabinoids.

Objective: To develop a comprehensive chromatographic method suitable for a wide range of fentanyl variants.

Methods: Sample preparation, LC/MS method development and calibration.

Results: A method suitable for 20 of the current fentanyl products was developed using certified reference material solutions as calibrants.

Conclusion: The method developed provides full traceability and uncertainty of measurement for optimum accuracy.

P8: The Effect of Warfarin on Calliphora Vomitoria Larvae Development & Semi-Quantitative GC-MS Analysis

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Introduction: Forensic entomotoxicology has the potential for drug identification when other human samples are limited or unavailable. It relies upon xenobiotic accumulation within feeding insects from typically deceased individuals who have been administered a drug prior to death. Warfarin is the most widely used anticoagulant globally, prescribed for the prevention of thromboembolism disorders such as deep vein thrombosis, stroke and pulmonary embolism. This wide consumption of warfarin means the likelihood of the drug being encountered when toxicologically analysing insects is inevitable. *Calliphora vomitoria* also known as the blue bottlefly, part of the order *Diptera* is one of the most common species initially encountered on a corpse in the UK.

Objective: This research aims to determine whether varying concentrations of warfarin impacts the development of *Calliphora vomitoria* larvae and to determine whether warfarin levels in *Calliphora vomitoria* larvae can be correlated to drug concentrations in tissues eaten by the larvae.

Method: Three 250g portions of pig liver were spiked with sheep's blood in Alsever's solution containing warfarin producing spiked meat concentrations of 1.5mg/kg, 10mg/kg and 50mg/kg. Approximately 60 2nd instar *Calliphora vomitoria* larvae were allowed to feed on each spiked meat concentration. The effect of varying concentrations of warfarin was observed with data collected on the larval development rates until 144 hours after being applied to the spiked meat. Sample preparation from the larvae was carried out via freezing the larvae with liquid nitrogen, crushing the frozen larvae and then adding methanol. Ibuprofen was added as the internal standard. Solid-phase extraction (SPE) was carried out with 200mg CLEAN-UP C-30 extraction columns. All solutions were derivatised with 50µl of TMAH. Semi-quantification of warfarin from the larvae was attempted with gas chromatography-mass spectrometry using a Shimadzu GC-2010 Plus with a Zebron ZB5 column (30.0m x 0.25mm x 0.25µm film thickness) coupled with a Shimadzu GCMS–QP2010 SE, run in selected ion monitoring (SIM) mode.

Results: A significant statistical difference (confidence interval: 95%) was only observed in larvae after 96 hours with a *p*-value of 0.00216 for weight and a *p* -value of 0.013824 for length recorded. Larvae reared on the highest concentration of 50.00mg/kg were recorded as having the largest average weight and length after 96 hours. Warfarin could not be identified in the GC-MS results, although the ibuprofen internal standard was identified.

Conclusion: *Calliphora vomitoria* larvae appear to exhibit an increased development rate prior to 96 hours after consuming higher concentrations of warfarin, however significance was not found to be maintained in data collected after the initial 96 hours.

P9: The effects of diet and regular mixers on alcohol pharmacokinetics.

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Introduction: Consumption of alcoholic drinks with a 'diet' mixers is increasing. There are reports in the literature that, when compared to standard mixers, these alter the pharmacokinetics of alcohol. Possibly by causing more rapid stomach emptying.

Objective: The aim of this study was to evaluate breath alcohol concentrations and the rate of gastric emptying using ^{13}C Acetate in healthy volunteers following consumption of either sucrose containing or artificially sweetened alcoholic drinks.

Method: This was an open labelled two-way crossover study. On two occasions, the participants ingested alcohol, once with diet coke, and once with regular coke. Seven healthy volunteers were studied twice in randomized order. On each session, they drank a standardised volume of vodka (37.5 % ABV), prepared with either 'regular' coke including sugar, or 'diet' coke with artificial sweetener. Their breath alcohol concentrations were taken every 15 minutes during 3 hours with the lion alcolmeter® SD-400 and lion alcolmeter® 500. Simultaneously, their breath samples for gastric emptying measurement were taken in breath bags. These breath samples were analyzed with an IRIS® instrument to get $t_{1/2}$ and t_{lag} parameters.

Results: Both the peak breath ethanol concentration (440± 72.3 vs. 386± 62.9μg/L) and the area under the breath ethanol curve between 0 and 180 minutes (507.4±67.6 vs. 448.1± 50.8units) were greater with diet coke. Gastric half-emptying time and lag phase time were calculated from the ^{13}C percent dose recovery values measured by IRIS® instrument with nonlinear regression analysis. Gastric half-emptying time was less for the diet drink than the regular drink (86.3±7.8 vs. 88.4±17.1 min) but t_{lag} was greater for the diet drink than the regular drink (48.1±9.6 vs. 46.1±15.7 min).

Conclusion: This study emphasizes that elements other than just the alcohol content of a drink should be taken into consideration in bearing in mind safe quantities of ingestion and the possibility for intoxication. The lack of sucrose in diet mixers might result in quicker gastric emptying of alcohol, causing an increase in its absorption rate into the blood, producing larger peak inebriation and greater exposure to various alcohol related hazards.

P10: Benzodiazepines: Are there "New Kids On The Block"? A survey of their prevalence in opioid substitution patients using LC/MS.

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HSE National Drug Treatment Centre

Introduction Benzodiazepines have a range of clinical uses being among the most commonly prescribed medicines globally and are commonly prescribed to patients attending our service. Over the last decade, the EU Early Warning System on new psychoactive substances (NPS) has detected an increasing number of new benzodiazepines that have appeared on Europe's drug market[1]. In 2016 the Forensic Science Ireland detected the new Benzodiazepine drugs Phenazepam, Nitrazolam, Etizolam and Chlorodiazepam in seizures tested[2]. Etizolam was implicated in, or potentially contributed to, the cause of death in 299 drug-related deaths in Scotland in 2017[3]. We obtained additional reference standards to enable detection of some of the new benzodiazepine drugs in use.

Objective To develop a multi-residue LC/MS method to test for 30 benzodiazepines or metabolites in urine including some new benzodiazepines (NPS) and to determine the profile of benzodiazepine drugs currently used in the addiction population.

Methods Building on a previously developed method which included 20 drugs, 10 new drugs including Etizolam, Estazolam, Phenazepam, Clonazolam, Pyrazolam, Deschloroetizolam, Flubromazepam, Flunitrazolam, Diclazepam, Demoxepam and Lormetazepam were tuned and added to the method. Separation and mass spectral conditions were optimised to allow unambiguous determination of 30 drugs. The method was successfully developed and put into routine use. Urine samples are subjected to hydrolysis with a purified β-glucuronidase called Abalonase™ which uses a much shorter hydrolysis time than traditional β-glucuronidase. This is followed by centrifugation, dilute n' shoot sample preparation and analysis by LC/MS. Urine samples were obtained from participants attending our service who are monitored on a regular basis for drug and alcohol use. 200 samples from patients attending our service which tested positive for Benzodiazepine class drugs by immunoassay screening were subjected to confirmatory analysis for Benzodiazepines to determine the profile of drugs present and to see if Etizolam or other new Benzodiazepines are being used. Results were recorded.

Results A multi-residue LC/MS method to test for 30 benzodiazepines or metabolites in urine including some new benzodiazepines (NPS) was successfully developed.Since the new method was initiated, a sample where Benzodiazepine identification was requested was positive for Etizolam, Nordiazepam, Temazepam, Oxazepam, Alprazolam, α-Hydroxyalprazolam and 7-Aminoclonazepam. This urine was also positive for Zopiclone by LC/MS and screened positive for Benzodiazepines, EDDP, Opiates and Cannabis by immunoassay screen. Drugs detected in a previous study were: oxazepam, temazepam, nordiazepam, 2-hydroxyethylflurazepam, alprazolam, a-hydroxyalprazolam, 7- aminoflunitrazepam and lorazepam. More comprehensive confirmatory analysis was conducted on 200 samples positive for benzodiazepine. Results of the current study will be presented and compared with previous findings.

Conclusion: In 2015 64% of poisoning deaths had poly drugs present (n=222) in 2015, benzodiazepines being the most common drug group implicated[4]. Using our method we detected Etizolam in a patient

urine sample along with 6 other benzodiazepines or metabolites with other drugs also present demonstrating an example of significant poly drug use.

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P11: Identification of gamma-butyrolactone (GBL) in forensic samples and related substances in alleged drug-facilitated sexual assault (DFSA) cases using benchtop nuclear magnetic resonance (NMR) spectroscopy

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Introduction: Gamma-hydroxybutyric acid (GHB) and gamma-butyrolactone (GBL) have been regularly reported as DFSA agents. GHB and GBL are also reported to be used in the club scene and are sold as 'liquid ecstasy'. These substances may also be used by bodybuilders as alternatives to steroids. In humans, GBL is rapidly converted by the liver to GHB. Alcohol and other psychoactive substances are known to enhance the sedative effects of these compounds. The forensic identification of GBL and GHB is complicated by the fact that GBL is very volatile. If the temperature increases or the pH decreases then this rapidly increases the conversion of GHB to GBL when these substances are in solution. Wet chemical tests have been used to identify GHB and GBL but these tests can be adversely affected if GBL or GHB are added to alcohol or other liquids. Other spectroscopic methods such as infrared (IR) and Raman spectroscopy may lack the specificity to unequivocally identify closely related compounds and if a mixture of substances is present then interpretation of the spectroscopic result may be difficult. Nuclear magnetic resonance (NMR) spectroscopy is an attractive analysis alternative. However, high-field NMR instruments are costly, may require specialist interpretation and are currently outside the reach of most forensic laboratories performing routine analyses for law enforcement purposes. The recent introduction of low-field bench-top NMR instruments has made the technique more affordable and thus more accessible. We report the use of bench-top NMR spectroscopy (1 H, 60 MHz) for the identification of GBL in forensic samples in alleged DFSA cases. A number of related compounds were also analysed to assess potential interferences.

Objective: To quickly and uniquely identify gamma-butyrolactone (GBL) and related substances in liquids as presented in forensic samples from cases where alleged DFSA was suspected using bench-top NMR spectroscopy.

Method: Samples in which liquids were suspected to be or contain GBL or GHB were analysed using an NMReady 60e NMR spectrometer (1 H, 60 MHz). Liquids were diluted with and solids dissolved in d6 DMSO for NMR analysis. Samples were run in 5 mm tubes at 32o C and varying numbers of scans were acquired to optimize spectra. Raw data was processed using Mnova software.

Results: A number of related compounds and precursors were analysed and they were found not to interfere with the unique identification of GBL. A key feature of the 1 H NMR spectrum for GBL was the triplet assigned to the CH2 adjacent to the ring oxygen as this provided a means for quantification. Further confirmation of GBL, was possible by performing two-dimensional NMR experiments such as 1 H1 H COSY. It was also possible to use this triplet in the 1H NMR spectrum when GBL was present along with ethanol (typical cases presentations).

Conclusion: Bench-top NMR spectroscopy proved to be a viable and reliable and quick method for the unique identification of GBL in forensic samples and for other forensic analyses where pure or relatively pure substances are submitted. Keywords (3): NMR, DFSA, forensic

P12: A sensitive GC-MS-MS method for the detection for THC and THCA in whole blood of drivers in Ireland

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Introduction: While medicinal cannabis is available in Ireland it is illegal to drive and be under the influence of THC. The Road Traffic Act in Ireland includes per se legislation for THC and THCA at 1ng/ml and 5ng/ml respectively in whole blood. This new measure was introduced in April 2017 and there is a medical exemption for medicinal cannabis users. The need for a sensitive quantitative method to enable enforcement and prosecution of the per se legislation was required. The MBRS also aim to carry out confirmation analysis on all drugs found positive on screening. The volume of sample available sometimes hinders this effort and increased instrument sensitivity may allow for the use of a reduced volume requirement for THC and THCA analysis in whole blood in the future

Objectives: The existing method was based on an Agilent 7000B Tandem MS and the objective was to evaluate the performance of the method on a 7010 Agilent Tandem MS.

Methods: The method involved preparation of a composite calibration curve for THC and THCA in blank blood including THC-D_3 and THCA-D_9 internal standards. The method was validated for the use of two specimen replicates of 1ml each. THC was extracted from whole blood using n-Hexane and extracts are transferred to clean silanised tubes following mixing and centrifugation. The resulting solvent phase was evaporated to dryness using N_2 and any THC in the residue was derivatised to THC-TMS after the addition if BSTFA (1% TMS) and incubation. Derivatised samples were evaporated to dryness and reconstituted in Butyl Acetate injection onto the Agilent 7010 Tandem MS.

Following the extraction of the THC fraction, specimens were buffered using citrate buffer. THCA was extracted using a Pentane: Ethyl Acetate mixture. The solvent phase was removed to a clean tube, evaporated to dryness and derivatised to THCOOH-2PFP using PFPA and PFPOH followed by an incubation period. Derivatised samples were injected onto the Agilent 7010 Tandem MS instrument for analysis.

Results: There was good correlation for THC data between samples analysed on the Agilent 7000B source and subsequently run on the Agilent 7010 instrument. However the response on the 7010 analyser was significantly higher. Response values were in the order of twenty times greater when analysed on the Agilent 7010 instrument when compared to original analysis in the Agilent 7000B system.

Conclusions: The 7010 instrument offered a significant increase in sensitivity. The MBRS currently aim to confirm all positive screening results, however this regularly poses a challenge due to the lack of whole blood specimen available. The increased sensitivity provided by the upgrade to the Agilent 7010 source will allow for the required reduction in aliquot volume going forward.

P13: Assessing the usefulness of solid-phase microextraction (SPME) for methadone and its metabolites: analysis in urine samples using HPLC/SPME fibre tips

Majidah Al fahmi and Dr. Calum Morrison

University of Glasgow

Introduction: To assess the usefulness of solid-phase microextraction (SPME) for methadone and its metabolites: analysis in urine samples using HPLC/SPME fibre tips. Solid-phase microextraction (SPME) has been introduced as a novel, simple and single-step technique for extracting methadone (MDN), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline (EMDP) from urine samples.

Methods: In this project, direct immersion SPME followed by high-performance liquid chromatography with ultraviolet detection (HPLC-UV) was developed to identify methadone and its metabolites. The separation was performed on a Gemini C18 (octadecyl carbon chain) analytical column (150 x 2.0 mm, 5 µm) and detected by an ultraviolet (UV) detector at 210 nm. The factors influencing the SPME procedure, such as the sample's pH, fibre coating type, salt addition and desorption solvent type were optimised.

Results: The best conditions were obtained with a C18 coating at a pH of 11, NaCl 15% and ethyl acetate. The recoveries of MDN, EDDP and EMDP under optimum conditions were 90.8%, 89.3% and 86.5 %, respectively. The calibration curves for urine samples showed good linearity under optimum conditions (R^2 range 0.9983–0.9988) in a concentration range of 0.025–4 µg/mL for the analytes, using hydrocodone as the internal standard. The selectivity of this method was evaluated and provided clean chromatograms with no interference in the analysis.

Conclusion: The results show that the novel SPME fibre tips have relatively high extraction efficiency for methadone and its metabolites.

All sponsoring vendors were asked to submit information about the products and services they can provide. These are included here for information purposes and are not an endorsement. Statements and views here are not those of UKIAFT but of each individual vendor.

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https://forensicrti.org/course/virtual-online-symposium-current-trends-in-forensic-toxicology-agilent/

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Tier 2

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- Industry leading UPLC, MS, informatics and chemistries

- A portfolio of complete solutions
- A team of dedicated scientists with extensive experience and understanding of forensic and toxicology applications

For more information please don't hesitate to get in touch

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